IMPROVEMENT OF MAIZE (*ZEA MAYS* L.) FOR HEAT STRESS TOLERANCE IN ZIMBABWE TO ENHANCE CLIMATE RESILIENCE IN NEW GENOTYPES

By

Ronica Mukaro

MScAgric. (Plant Breeding), University of Zimbabwe, Zimbabwe
BScHons. (Crop Science), Bindura University of Science Education, Zimbabwe

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

Discipline of Plant Breeding

School of Agricultural, Earth and Environmental Sciences

College of Agriculture, Engineering and Science

Republic of South Africa

February 2024

THESIS ABSTRACT

Maize (Zea mays L.) is an important crop for food security globally. Rising temperatures due to climate change have significantly affected production and productivity of maize in sub Saharan Africa (SSA). Global warming is predicted to increase the frequency, duration, and intensity of heat stress, but the region's ability to adapt to these changes is limited. Given the significant maize yield losses reported due to heat stress, the use of high yielding heat tolerant maize varieties offers a sustainable solution to these challenges. In Zimbabwe and SSA at large, breeding maize for heat stress tolerance is at infancy stage. Therefore, the objectives of this study were to i) determine the genetic potential of exotic heat stress tolerant maize donor lines (HSTDLs) obtained from CIMMYT-India and CIMMYT-Zimbabwe elite lines for potential use in sub-tropical breeding programs, ii) assess the genetic diversity and genetic purity of heat donor lines and elite CIMMYT-Zimbabwe lines using single nucleotide polymorphism (SNP) markers, and iii) determine the performance and yield stability of early maturing commercial hybrids currently on the market in Zimbabwe under heat stress and random drought and heat stress conditions.

In the first study, 14 HSTDLs from CIMMYT-India were crossed with 15 locally adapted elite lines from CIMMYT-Zimbabwe using the North Carolina Design II mating scheme. The successful 175 crosses were evaluated together with five commercial hybrids at two locations in the lowveld area of Zimbabwe during the 2020 winter season under managed heat stress and optimal conditions. The parental line trial was planted adjacent the hybrid trial at each location to determine the *per se* performance of the heat donor lines under heat and optimal conditions. The sentence was restructured and its now reading “ The design II analysis revealed significant ($p < 0.01$) general combining ability (GCA) and specific combining ability (SCA) effects for GYD under heat stress (HTS), optimal conditions (OC), and across locations an indication that GYD is controlled by both additive and non-additive gene action. Three HTSDLs (CAL14138, CAL152, and CAL1440) exhibited significant positive GCA effects for GYD under HTS conditions. The crosses DJ265-15 × VL1018816 and DJ267-9 × CAL1440 revealed positive significant SCA for GYD under HTS. The donor lines (HTSDLs) CAL14138, CAL152 and VL109126 exhibited superior *per se* performance under HTS, OC and across environments.

Thirty five inbred lines were genotyped to assess their genetic diversity, relatedness and purity using 94 single-nucleotide polymorphism (SNP) markers. The identity-by-state (IBS) genetic distance matrix revealed pairwise genetic distance among the inbred lines ranging from 0.04 to 0.64. The widest genetic distance was between inbred line pairs: CZL1112c and CZL16018; CAL14135 and ZL132077; and CZL15153 and CZL16018. The shortest genetic distance was between inbred lines CAL152 and CAL14138, CAL14138 and VL109126; and ZL132077 and DJ611-1. The neighbor-joining algorithm grouped inbred lines into three different main clusters. Some heat tolerant donor lines clustered together with local lines, while the other cluster consisted of either CIMMYT-Zimbabwe or CIMMYT- India lines. Majority (85.78%) of lines assessed were genetically pure with less than 5% heterozygosity. About 54.28% of the inbred lines evaluated exhibited 100% genetic purity.

In the third study, 20 early maturing commercial hybrids and 5 experimental hybrids were evaluated across six locations during the 2020/21-2021/22 seasons for adaptability and stability under heat stress, random drought and heat stress conditions. The genotype main effect plus genotype x environment interaction (GGE) biplot showed that commercial hybrids G3 (4.79 t ha^{-1}), G20 (3.99 t ha^{-1}) and G22 (4.09 t ha^{-1}) were the most adapted under HTS condition while experimental hybrid, G4 (4.31 t ha^{-1}), was the most adapted under HTS conditions. Under random drought and heat stress (RDHS) conditions, the most adapted commercial hybrids were G12 (4.66 t ha^{-1}), G14 (4.39 t ha^{-1}), G15 (4.02 t ha^{-1}), G13 (4.17 t ha^{-1}), G21 (3.76 t ha^{-1}) and G25 (3.50 t ha^{-1}). The ‘ranking’ GGE biplot identified commercial hybrids G16 (4.94 t ha^{-1}) and G3 (4.79 t ha^{-1}) as high yielding and stable across stress and non-stress conditions. The experimental hybrids G6 (4.77 t ha^{-1}) and G7 (4.67 t ha^{-1}) were stable across environments. The experimental genotype, G8 (5.47 t ha^{-1}), was overally, the highest performing but was unstable.

The exotic lines that exibited significant positive GCA can be exploited for introgression of heat tolerant genes into local maize populations in breeding for heat stress tolerance. The availability of genetic diversity and the relationship observed among the HSTDLs and the local lines shows that there are valuable heat tolerant inbred lines within CIMMYT- Zimbabwe programme that could be used in heat stress tolerance breeding in SSA. Generally the study shows the potential of breeding for heat stress tolerance in SSA.

DECLARATION 1 – PLAGIARISM

I, **Ronica Mukaro**, declare that,

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

Signed:

[redacted]

Ronica Mukaro

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

[redacted]

Professor Julia Sibiya (Supervisor)

Dr. Casper. Nyaradzai Kamutando (Co-supervisor)

Dr. Cosmos Magorokosho (Research work supervisor)

DECLARATION 2 - PUBLICATIONS AND MANUSCRIPTS

Chapter 3 of this thesis has already been published in a peer reviewed journal. The published manuscript, authors and publication journal details read as follows:

1. **Mukaro, R**., Kamutando, C.N., Magorokosho, C., Mutari, B., Zaidi, P.H., Kutywayo, D. and Sibiya, J. Genetic Potential of Tropically Adapted Exotic Maize (*Zea mays* L.) Heat-Tolerant Donor Lines in Sub-Tropical Breeding Programs. Agronomy, 2023, 13, 2050. https://doi.org/10.3390/agronomy13082050

ACKNOWLEDGEMENTS

I would like to extend my profound and sincere gratitude to the following people and organizations for their invaluable contribution to the successful completion of this work.

- My main supervisor Professor Julia Sibiya for her tireless and passionate supervision, kind guidance, unwavering support, inspiration as well as securing tuition fees throughout my study period. Working with her was a blessing.
- My co-supervisor from University of Zimbabwe, Department of Plant Production Sciences and Technologies, Dr. Casper Nyaradzai Kamutando for his encouragement, supervision and guidance in data analysis and manuscript writing.
- My co-supervisors at CIMMYT Dr. Cosmos Magorokosho for his inspirational supervision, guidance, mentorship, and support with field research funding
- Special thanks to Andile Etah Mshengu and Tamlyn Skye for their tireless efforts in helping with administrative issues.
- I am also thankful to Professor Edmore Gasura for encouraging me to apply for the program, you are appreciated Prof.
- My sincere gratitude goes to the CIMMYT Zimbabwe Breeding Team: Toverengwa Chitana, Stanley Gokoma, Semai Viola, Irene Viola, Amin Mataka and Passmore Kasere for the various contributions they made during the study.
- My thanks also go to Dr. Zamman-Alla Mainassara for assistance in weather data collection.
- The technical staff of the Agronomy Research Station, Chisumbanje and Save Valley Experiment Stations, are thanked for making my fieldwork go more smoothly than anticipated. These include Mr. Chiwade, Ms. Muzondo, Ms. Matimba, and Mr. C. Chohunoita. Thank you so much!
- I would also want to express my gratitude to the Department of Research and Specialist Services (DR&SS), which is led by Dr. Dumisani Kutywayo and Ms. Busiso Olga Mavankeni, for providing me with the time I needed to complete my studies.

- I also want to thank my fellow students Lucia Ndlala, Fortunate Makore, Nakai Matongera, Prince Matova, Barbra Chisikaurayi, Purity Mazibuko, Tinovonga Gonhi and Rejoice Nyoni, for their input and support during the study period
- Special mention goes to Bruce Mutari and Aleck Kondwakwenda for their support and the extra time we spent together online advancing the cause.
- I also appreciate moral support from my family: Tapiwa, Leatrice, Pardon, Janet, Luke, Linda and my boy Prince.
- Special thanks go to Misheck Musokwa who for the first time took me around KwaZulu Natal University.
- SEED-Co Kadoma is thanked for hosting random drought and heat stress trials.
- I am truly grateful to those who contributed directly or indirectly to this work but were not duly mentioned, may God bless you.
- Finally, I thank God for giving me the grace and strength to complete the studies.

DEDICATION

To my late parents Vimbai Jennifer Chamisa-Mukaro and Edmore Mukaro who could not live to see this adventure.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS AND ACRONYMS

ASI	Anthesis-Silking Interval
AD	Anthesis date
AFLP	Amplified fragment length polymorphism.
AGG	Accelerating Genetic Gains
AGD-R	Analysis of Genetic Designs in R
ANOVA	Analysis of variance
BLUPs	Best linear unbiased predictions
CIMMYT	International Maize and Wheat Improvement Center
CRS	Chiredzi research Station
CRISPR	Clustered regularly interspaced short palindromic repeats.
Cas9	CRISPR associated protein 9.
DF	Degrees of freedom
DH	Doubled haploid.
DNA	Deoxyribonucleic acid
DTMA	Drought tolerant maize for Africa
EH	Ear height.
ESA	Eastern and Southern Africa
GCA	General combining ability.
$GCA_{f,}$	General combining ability -female.
GCA_m	General combining ability- male.
GEI	Genotype by environment interaction
GEBV	Genomic estimated breeding values
GE	Genome editing
GGE	Genotype-genotype x environment
GMP	Global maize program

GYD	Grain yield
GS	Genomic selection
GWAS	Genome-wide association study
H^2	Broad sense heritability
Ha	Hectares
HSPs	Heat shock proteins
HSFs	Heat shock factors
HSTDLs	Heat Stress tolerant donor lines
HRS	Harare Research Station
HTMA	Heat Tolerant Maize for Asia
IBS	Identity-by-state
IITA-MIP	International Institute of Tropical Agriculture-Maize Improvement program
IITA	International Institute of Tropical Agriculture
IMIC	International Maize Improvement Consortium
KASP	Kompetitive allele-specific polymerase chain reaction
KRS	Kadoma Research station
LSD	Least significant differences
Masl	Metres above sea level
MGYP	Mean grain yield performance.
MS	Mean sum of squares
META-R	Multi-Environment Trials Analysis in R
MEGA	Molecular evolutionary genetics analysis
MRS	Matopos research station
MT	Metric tonnes
PES	Panmure Experiment Station
PSII	Photosystem II
QTL	Quantitative trait loci

RAPD	Random amplified polymorphic DNA
RDHS	Random drought and heat stress
Rep	Replication
REML	Restricted maximum likelihood.
RFLP	Restriction fragment length polymorphism
RH	Relative humidity
SCA	Specific combining ability
SSRs	Simple sequence repeats
STMA	Stress Tolerant Maize for Africa
SS	Sum of squares
SSA	Sub Saharan Africa
SNP	Single-nucleotide polymorphism
TASSEL	Trait Analysis by Association Evolution and Linkage
TALENs	Transcription activator-like effector nucleases
USAID	United States Agency for International Development
VPD	Vapor-pressure deficit
ZFN	Zinc finger nuclease

CHAPTER 1

Introduction to thesis

1.1 Importance of maize in sub Saharan Africa

Maize (Zea mays L.) is the third most important crop in the world in terms of production area, volume, and the percentage of the people that depends on it as a staple food (Ranum et al. 2014; Zaidi et al. 2020). Globally, maize is grown on an estimated area of 193.7 million hectares (ha) with approximate yield of 1147.7 million MT (FAO, 2021). The crop is grown at various latitudes and altitudes signifying its ability to thrive in diverse environments (Badu-Apraku and Fakorede, 2017). Additionally, maize has exceptional production potential among cereal crops (Badu-Apraku and Fakorede, 2017; Tiwari and Yadav, 2019) making it appealing to farmers in locations with limited land and severe population pressure (Shiferaw et al. 2011). In terms of nutrients, maize has about 72% starch, 10% protein, 4% fat, 8.5% fibre, 3 % sugar, 1.7% ash, vitamin B complex, and has an energy mass of 365 Kcal/100g (Ranum et al. 2014).

Maize is a multi-purpose crop, that is used for human food, animal feed, industrial applications and bio-energy production (Chen et al. 2017; Huma et al. 2019). The crop has been extensively used in various studies including plant genetics, plant physiology, soil fertility, biochemistry and other fields of studies (Bai, 2003). In sub Saharan Africa (SSA), maize production accounts for 40% of crops grown and more than 80% of maize produced is used for food, supplying about 30% of the total calories consumed, with daily consumption ranging from 52 to 450 g per person (Prasanna et al. 2021). Maize is also a source of revenue for farmers and a food security crop in SSA (Erenstein et al. 2022). In Zimbabwe, maize contributes approximately 14% to the agricultural gross domestic product of the country https://www.fao.org/zimbabwe/fao-in-zimbabwe/zimbabwe-at-a-glance/en/. Successful and continuous production of maize in SSA is very important and any interruption in the supply at any level has negative impact on the vulnerable smallholder farmers and industrial raw material supplies.

1.2 Maize production in sub-Saharan Africa (SSA)

In SSA, maize is primarily grown by resource–poor smallholder farmers in marginal areas as a rain-fed crop (Prasanna et al. 2021). Extreme weather events are usually felt by famers and have negative impact on them because they are less able to adapt, and this makes rain-fed crops particularly vulnerable. Production of maize is increasing and the increase is linked to the significant expansion of production area (187%) rather than maize yields (Cairns and Prasanna, 2018) and this is very unsustainable. In Zimbabwe, maize is ranked the highest in terms of total production and area grown (Mutari et al. 2021). It is produced on nearly 1million hectares, which is more than 70% of the country's total area used for cereal production (FAO, 2023). With the exception of drought years, Zimbabwe was producing maize in excess before 2000 (Mutambara, 2016). Thereafter, the yield levels dropped to less than 1.0 MTha^{-1} but the land reform initiatives allowed the production area to keep growing (Mutambara, 2016; FAO, 2021) and this is very bad for wildlife habitats.

1.3 Maize production constraints in SSA

The average maize grain yield in SSA region is around 1.5 MTha^{-1}, which is almost 20% less than the average yield in developed countries (FAO, 2019; Prasanna et al. 2021). The low yields are attributed to the prevalence of a vast number of biotic and abiotic stresses and their interaction as well as the socio-economic constraints (Shiferaw et al. 2011; Cairns et al. 2013a; Frey et al. 2016). The biotic factors include diseases such as Maize streak virus (*Cicadulina mbila*), Gray leaf spot (*Cercospora zea-maydis***)** and Turcicum leaf blight (*Exserohilum turcicum*) and the insect pests such as maize stalk borers (*Busseola fusca*), fall armyworm (*Spodoptera frugiperda*), larger grain borer (*Prostephanus truncates*), and maize grain weevil (*Sitophilus zeamais*). Abiotic stressors such as low soil fertility, drought, and heat stress have been linked to considerable yield loss in maize (Sibiya et al. 2012; Masuka et al. 2017). The cost of inputs is a major socio-economic factor affecting maize productivity and production in SSA (Sibiya et al. 2012).

Climate change-related stresses were reported to have bigger impact on maize productivity than any other constraints (Masuka et al. 2017; Jodage et al. 2017; Kamutando et al. 2018; Thayil et al. 2020; Zaidi et al. 2023). Among climate related stress factors, heat and drought stresses have been recognized as the most significant and usually co-occurring (Worku et al. 2016; Prasanna et al. 2021). Although these two stresses are correlated and have been repeatedly reported in SSA, heat stress due to climate change has become more important (Tesfaye et al. 2017). Under heat stress, an increase in VPD results in greater water requirements for evapotranspiration, plants suffer from physiological drought along with high- temperature stress (Zaidi et al. 2023) an indication that heat stress is more destructive than drought stress. Compared to increased intra-seasonal fluctuation in precipitation, rising temperatures cause larger loss in maize yields (Rowhani et al. 2011). According to Lobell et al. (2011) maize yields are lowered more when temperatures rise by 2°C than when precipitation drops by 20%.

1.4 Heat stress as major constraint to maize production

Heat stress is one of the main abiotic stressors linked to severe food shortages in SSA, Asia and Latin America (Cairns et al. 2012; 2013b; Nelimor et al. 2019; Zaidi et al. 2023). A significant drop in maize production due to heat stress was reported in several maize-growing areas of the world (van der Velde et al. 2010). Heat stress primarily accelerate phenology, limits biomass production and shortens reproductive phase on maize crop resulting in severe yield losses (Fahad et al. 2017; Sabagh et al. 2020). On the other hand, heat stress is most devastating when it occurs at flowering stage (Meseka et al. 2018; Zaidi et al. 2020) causing yield losses higher than 45% (Sultan and Gaetani, 2016; Fahad et al. 2017).

In SSA, maize yield losses due to heat stress range from 1% to 100% depending on the severity, duration of the stress, the rate at which the temperature rises, and the growth stage at which the crop is subjected to the stress (Ruelland and Zachowski, 2010; Kaushal et al. 2016; Fahad et al. 2017). For every degree day spent above 30°C, the final yield of maize in Africa was reported to decline by 1% (Lobell et al. 2013). Khodarahmpour et al (2011) reported grain yield reduction of up to 70% under heat stress condition in maize inbred lines. During 2015-16 season, high temperature together with El Niño were reported as the biggest yield reducing factors in many areas of Zimbabwe and this left a lot of people relying on food aid (World Food Program, 2016). Projections are showing that by 2050, about 45% of the world's maize-producing region will experience temperatures above 35^0C for five days on average per year during the reproductive stage. Given that maize yield is predicted to decrease by 3%–13% with a mere 1^0C increase in the mean seasonal temperature, heat stress becomes an area of concern to the scientists (Lobell et al. 2011; Tesfaye et al. 2017).

1.5 Heat stress tolerance breeding strategies in maize

Heat stress is a quantitative trait whose inheritance is controlled by many genes with small effect (Frey et al. 2016). Understanding the genetic and physiological mechanism of the maize plant is critical to develop the best breeding strategy for heat stress tolerance in maize. Projected climatic and environmental changes require breeding strategies that deliver both substantial increase in grain yield potential and tolerance to extreme weather conditions. The effective strategies that can be used in breeding for heat tolerance include; (i) introgression and incorporation of heat tolerant genes into adapted maize germplasm based on the assumption of a linear relationship between genetic divergence of the involved populations (Holland, 2004; Goodman, 1999), (ii) selection for yield under optimal conditions and evaluation in replicated multi-location trials with variable temperature regime with the postulation that genes for heat tolerance are present in elite high yielding material (Bai, 2003; Zaidi et al. 2020), and (iii) screening germplasm for yield and related secondary traits under heat stress conditions with the assumption that maize yields are usually very low and wide environmental variances affect the performance of the test genotypes (Blum, 1988; Jodage et al. 2017).

Progress in heat stress tolerance breeding has been slow due to genotype x environmental interactions (G x E) caused by year-to-year fluctuations in the timing and intensity of heat stress in field breeding nurseries. Indirect selection for grain using secondary traits under heat stress conditions can improve the efficiency of breeding for heat-tolerant genotypes. The use of secondary traits helps to overcome the challenges associated with direct selection for high grain yield under elevated temperature like low heritability of grain yield, which is due to the small genetic variance and the occurrence of poorly understood G x E (Maazou et al. 2016). Maize breeders have resorted to the use of secondary traits such as leaf firing, tassel blasting, anthesis-silking interval and leaf senescence that are significantly correlated with grain yield under heat stress, but have not yet fully exploited the selection gains from these traits. In addition, the use of phenotypic selection can be minimized by the use of molecular markers, which enables evaluation of breeding materials at least twice a year, hence expediting the breeding process (Alam et al. 2017).

1.5 Rationale of the study

Heat stress has become a significant constraint to maize production in SSA. The stress has caused high yield losses and has important implication on food supply and security in the region and Zimbabwe

(Cairns et al. 2013a). Despite significant yield losses recorded in SSA due to heat stress, climate projections have shown a rise in temperature and higher frequency of extreme events (FAO, 2021). A rise in global mean temperature by 0.3^{o}C per decade is anticipated to increase the current values by approximately 1^{o}C and 3^{o}C by 2025 and 2100, respectively (FAO, 2021). Given the cumulative evidence of elevated temperatures due to climate change and its impact in maize production in SSA, breeding for heat tolerance becomes a priority in both public and private breeding programs. The use of climate-resilient germplasm has the potential to increase yield by 5–25% in Africa (Cairns and Prasanna, 2018). Therefore, it is necessary to breed maize for heat stress tolerance in SSA to improve yield levels in the light of climate change.

Unpublished research conducted by CIMMYT - Zimbabwe has shown that there is limited genetic diversity for heat stress tolerance in Zimbabwe maize local germplasm (Magorokosho unpublished 2016). Introgression of preferred genes into locally adapted germplasm is regarded as the primary step in the development of improved germplasm (Chen et al. 2012). Considering the heat stress challenges experienced in Zimbabwe and SSA at large and the fact that local germplasm is not able to provide sufficient genetic variability to breed for heat tolerance, CIMMYT-Zimbabwe sourced exotic heat tolerant maize donor lines from India to use in heat stress tolerance breeding. The breeding potential of these heat tolerant donor lines in SSA breeding programs is not known. It is, therefore, important to establish the breeding value of the exotic lines to enable identification of good parents to use in initiating heat stress tolerance breeding program. Additionally, it is important to establish the genetic relationship between the heat tolerant donor lines and local lines as the information is important in designing the heat tolerant breeding strategy.

Priorities in tropical maize breeding programs were previously focused on abiotic stresses, such as drought, and poor soil nitrogen, but not breeding for heat stress tolerance. However, heat stress tolerance has become an important trait in many breeding programs in SSA. In Zimbabwe, the national maize breeding program, and private seed companies have developed and commercialized several high yielding maize hybrids with good adaptability to wider environments but these varieties were not bred for heat stress tolerance. Considering the time-lag between development, evaluation, registration, and adoption of new varieties, it is prudent to assess the performance of commercial locally available maize varieties currently on the market under heat stress to identify those that can confer heat stress tolerance so that they can be recommended for production in heat stress prone areas. The use of varieties with increased tolerance to heat stress can offset the predicted yield declines in heat prone areas (Cairn et al. 2013a).

1.6 Objectives and hypothesis

1.6.1 General Objective

The study aims to introgress heat tolerant genes into CIMMYT-Zimbabwe maize germplasm to enhance climate resilience in new maize varieties in SSA.

1.6.2 Specific objectives

The specific objectives of this study were:

i. To determine the genetic potential of exotic heat tolerant maize donor lines from India and CIMMYT-Zimbabwe elite inbred lines.

ii. To assess the genetic relatedness, and purity of heat tolerant maize donor lines from India and CIMMYT Zimbabwe elite inbred lines using SNP markers.

iii. To determine the performance and yield stability of early maturing commercial and experimental hybrids under heat stress prone areas of Zimbabwe.

1.6.3 Research hypotheses

The hypotheses tested include:

i. CIMMYT-Zimbabwe maize elite lines and Indian heat tolerant maize donors have good combining ability effects for heat stress tolerance and related phenotypic traits.

ii. Maize heat tolerant donor lines from India and elite lines from Zimbabwe are genetically different, exhibiting distinct levels of genetic diversity, relatedness, and purity as measured by the Single nucleotide polymorphism (SNP) markers.

iii. Among the early maturing commercial and heat tolerant experimental hybrids available in Zimbabwe there are high yielding and stable hybrids that can perform reasonably well under heat stressed conditions.

1.7 Thesis structure

This thesis consists of six chapters, including one introductory chapter (Chapter 1), one review chapter (Chapter 2), three experimental chapters (Chapters 3-5) and an overview chapter (Chapter 6). One of the three experimental chapters has already been published. Chapters 2-5 are independent and/or intend to be published, as such there are some overlaps and unavoidable repetitions of references and some introductory information between chapters. Additionally, the requirements for figure preparation varies from one journal to another, figure panel numbering style may vary from one chapter to another but has been kept consistent within each chapter.

Chapter 1 contains the general introduction, rationale of the study, objectives of the study, the hypothesis, and outline of the thesis structure.

Chapter 2 contains the literature review, which discusses the importance of heat stress on maize and identifies the current knowledge and the knowledge gaps in breeding for heat stress tolerance in maize in SSA.

Chapter 3 has been published in the journal of agronomy, https:// doi.org/10.3390/agronomy13082050. The chapter presents the genetic potential of tropically adapted exotic maize (*Zea mays L.*) heat tolerant donor lines in sub-tropical breeding programs.

In **Chapter 4,** SNP markers were employed to determine the genetic diversity, relatedness and genetic purity of CIMMTY-India heat donor lines and CIMMYT-Zimbabwe elite lines.

Chapter 5 presents the evaluation of early maturing commercial hybrids and heat tolerant experimental hybrids currently on the market in Zimbabwe and heat tolerant experimental hybrids under heat stress, random drought, and heat stress conditions to identify those that are high yielding and have some degree of tolerance to heat stress to recommend for use in stress prone areas.

Chapter 6 is the last and presents the overall discussion of the thesis and analyses the implications of the thesis findings.

References

Alam, A.; Seetharam, K.; Zaidi,P.H.; Dinesh, A.; Vinayana, T.M.; Nathb, U.K. Field Crops Research Dissecting heat stress tolerance in tropical maize (Zea mays L .) *Field Crops Res*. 2017, 204, 2011–2013, https://doi.org/10.1016/j.fcr.2017.01.006

Badu-Apraku, B.; and Fakorede, M.A.B. Advances in genetic enhancement of early and extra-early maize for sub-Saharan Africa. 2017. Adv. Genet. Enhanc. 2017, 1–604. doi: 10.1007/978-3-319-64852-1.

Bai, J. Genetic variation of heat tolerance and correlation with other agronomic traits in maize (*Zea mays L.*) recombinant inbred line population. 2003, Texas Techinical University, Masters Desertation

Blum, A. Plant breeding for stress environments. Heat resistance in plants. 1986

Cairns, J.E.; Sonder, K.; Zaidi, P.H.; Verhulst, N.; Mahuku, G. Maize Production in a changing climate : Impacts , Adaptation , and Mitigation Strategies. *Adv. Agron*. 2012, 114: 1–58. doi: 10.1016/B978-0-12-394275-3.00006-7.

Cairns, J. E.; Crossa, J.; Zaidi, P.H.; Grudloyma, P.; Sanchez, C.; Araus, J.L., Thaitad, S.;Makumbi, D.; Magorokosho, C.; Bänziger, M.; Menkir, A.; Hearne , S.; Atlin, G. N. Identification of drought, heat, and combined drought and heat tolerant donors in maize. *Crop Sci*, 2013a 53(4), 1335–1346. doi: 10.2135/cropsci2012.09.0545.

Cairns, J.E.; Hellin, J.; Sonder, K.; Araus, J.L.; MacRobert, J.F.; Thierfelder, C.; Prasanna, B. M. Adapting maize production to climate change in sub-Saharan Africa. *Food Secur*. 2013b 5(3): 345–360. doi: 10.1007/s12571-013-0256-x.

Cairns, J.E.; Prasanna, B.M. Developing and deploying climate-resilient maize varieties in the developing world. *Curr. Opin. Plant Biol*. 2018, 45: 226–230. doi: 10.1016/j.pbi.2018.05.004.

Chen, K.; Camberato, J.J.; Vyn, T.J. Maize grain yield and kernel component relationships to morphophysiological traits in commercial hybrids separated by four decades. *Crop Sci.* 2017, 57(3): 1641–1657. doi: 10.2135/cropsci2016.06.0540.

Chen, J.; Xu, W.; Velten, J.; Xin, Z.; Stout. J. Characterization of maize inbred lines for drought and heat tolerance. *J. Soil Water Conserv.* 2012, 67(5), 354–364. doi: 10.2489/jswc.67.5.354.

Chen, J.; Xu, W.; Velten, J.; Xin, Z.; Stout, J.; Erenstein, O.; Jaleta, M.; Sonder, K.; Mottaleb, and Prasanna, B.M. Global maize production, consumption and trade: Trends and research and development implications. *Food Secur*. 2022, doi: 10.1007/s12571-022-01288-7.

Erenstein, O.; Jaleta, M.; Sonder, K.; Mottaleb, K.; Prasanna, B. M. Global maize production, consumption and trade: trends and Research and Development implications. *Food Sec. 2022*, 14, 1295–1319. https://doi.org/10.1007/s12571-022-01288-7

Fahad, S.; Bajwa, A.A.; Nazir, U.; Anjum, S.A.; Farooq, A.; Zohaib, A.; Sadia. S.; Nasim, W.; Adkins, S.; Saud, S.; Ihsan, M.Z, Alharby, H.; Wu, C.; Wang, D.; Huang, J. Crop production under drought and heat stress: Plant responses and management options. *Front. Plant Sci*. 2017, 8, 1147, doi: 10.3389/fpls.2017.01147.

FAO, Cereals Agricultural Outlook 2018-2027. Oced/Fao: 109–126. https://www.oecdilibrary.org/agriculture-and-food/oecd-fao-agricultural-outlook-2018-2027_agr_outlook-2018-en, 2018, Accessed 28 August 2023.

FAO, Global Information and Early Warning System on Food and Agriculture, 2019. https://www.fao.org/giews/en/ Accessed 20/9/2021

FAO, Statistical Yearbook, World Food and Agriculture ,2021. https://www.fao.org/3/cb4477en/cb4477en.pdf : Accessed 10/23/21

FAO, Statistical database of the Food and Agriculture of the United Nations (Rome: FAO), 2023,Available at: https://www.fao.org/faostat/en/#data/QCL (Accessed 2 Feb 2023).

Frey, F.P.; Presterl, T.; Lecoq, P.; Orlik, A.; Stich. B. First steps to understand heat tolerance of temperate maize at adult stage: identification of QTL across multiple environments with connected segregating populations. *Theor. Appl. Genet*. 2016, 129(5): 945–961. doi: 10.1007/s00122-016-2674-6.

Goodman, M.M. Broadening the genetic diversity in maize breeding by use of exotic germplasm. *Genet. Exploit. Heterosis Crop*. 1999, 139–148. doi: 10.2134/1999.geneticsandexploitation.c13.

Holland, J.B. Breeding: Incorporation of exotic germplasm. *Encyclopedia of Plant and Crop Science. Marcel Dekker,* 2004, 222-224: doi=e597fc3d3b6389b03e1b257d4bf9674dd6c540b9

https://www.fao.org/zimbabwe/fao-in-zimbabwe/zimbabwe-at-a-glance/en/.

Huma, B.; Hussain, M.; Ning, C.; Yuesuo. Y. Human Benefits from Maize. *Sch J Appl Sci Res*. 2019, 2 (2), 4-7

Jodage, K.; Kuchanur, P.H.; Zaidi, P.H.; Patil, A.; Seetharam, K.; Vinayan, M.T.; Arunkumar, B. Genetic Analysis of Heat Stress Tolerance and Association of Traits in Tropical Maize (Zea mays L.). *Environ. Ecol*. 2017, 35(3C): 2354–2360.

Kamutando, C.N.; Magorokosho, C.; Dari. S. Combining Ability for Grain Yield Performance among CIMMYT Germplasm Adapted to the Mid-Altitude Conditions. *J. Agric. Sci*. 2018, 10(3): 253. doi: 10.5539/jas.v10n3p253.

Kaushal, N.; Bhandari, K.; Siddique, K.H.M .; Nayyar. H. Food crops face rising temperatures : An overview of responses, adaptive mechanisms, and approaches to improve heat tolerance *Cogent Food Agric.* 2016, 28(1). doi: 10.1080/23311932.2015.1134380.

Khodarahmpour, Z.; Choukan, R.; Bihamta, M.R.; Hervan. M. Determination of the best heat stress tolerance indices in maize (Zea mays L.*)* inbred lines and hybrids under Khuzestan Province conditions. *J. Agric. Sci. Technol.* 2011, 13(1): 111–121.

Lobell, D.B.; Bänziger, M.; Magorokosho, C.; Vivek. B. Nonlinear heat effects on African maize as evidenced by historical yield trials. *Nat. Clim. Chang.* 2011, 1(1): 42–45. doi: 10.1038/nclimate1043.

Lobell, D.B.; Hammer, G.L.; McLean, G.; Messina, C.; McRoberts, M.J.; Schlenker, W. The critical role of extreme heat for maize production in the United States. *Nat. Clim. Chang.* 2013, 3(5): 497–501. doi: 10.1038/nclimate1832.

Maazou, A.S.; Tu, J.; Qiu, J.; Liu, Z.Z. Breeding for Drought Tolerance in Maize (Zea mays L.) *Am. J. Plant Sci.* 2016, 7, 1858–1870. doi: 10.4236/ajps.2016.714172.

Masuka, B.; Atlin, G.N.; Olsen, M.; Magorokosho, C.; Labuschagne, M.; Crossa, J.; Bänziger, M.;

Pixley, K.V.; Vivek, B.S.; Von Biljon, A.; Macrobert, J. Gains in maize genetic improvement in Eastern and Southern Africa: I. CIMMYT hybrid breeding pipeline. *Crop Sci.* 2017. *57*(1), 168-179. https://doi.org/10.2135/cropsci2016.05.0343.

Meseka, S.; Menkir, A.; Bossey, B.; Mengesha, W. Performance assessment of drought tolerant maize hybrids under combined drought and heat stress. *J. Agron*, 2018, 8(12).1-17, doi: 10.3390/agronomy8120274.

Magorokosho, C. unpublished . Evaluation of local maize inbre lines and hybrids under heat stress conditions, 2016.

Mutambara, J. Usaid Strategic Economic Research and Analysis – Zimbabwe (Sera) Program: Maize Production and Marketing in Zimbabwe : Policies for a high growth strategy. USAID Strateg. Econ. Res. Anal. Zimbabwe Progr. 2016,: 66. http://pdf.usaid.gov/pdf_docs/PA00MDKC.pdf.

Mutari, B.; Sibiya, J.; Bogweh N. E.; Simango, K.; Gasura, E. Farmers' perceptions of navy bean (*Phaseolus vulgaris L*.) production constraints, preferred traits and farming systems and their implications on bean breeding: A case study from South East Lowveld region of Zimbabwe. *J. Ethnobiol. Ethnomed. 2021,* 17(1): 1–20. doi: 10.1186/s13002-021-00442-3.

Nelimor, C.; Badu-Apraku, B.; Tetteh, A.Y.; N'guetta, A.S.P. Assessment of genetic diversity for drought, heat and combined drought and heat stress tolerance in early maturing maize landraces. *J. Agron* . 2019, 8, 518, doi: 10.3390/plants8110518.

Prasanna, B.M.; Cairns, J.E.; Beyene, Y.; Zaidi, P.H.; Makumbi, D.; Gowda, M.; Magorokosho, C.; Zaman-Allah, M.; Olsen, M.; Das, A.; Worku, M.; Gethi, J.; Vivek, B.S.; Sudha, K.; Rashid, Z.N.; Vanayan, M.T.; Issa, A.B.; Vicente, F.S.; Dhliwayo, T.; Zhang, X. Beat the stress: breeding for climate resilience in maize for the tropical rainfed environments *Theor. Appl. Genet*. 2021, 134(6), 1729–1752, doi.org/10.1007/s00122-021-03773-7

Ranum, P.; Peña-Rosas, J.P.; Garcia-Casal, M.N. Global maize production, utilization, and consumption. *Ann. N.Y. Acad. Sci*. 2014, 1312(1), 105–112. doi: 10.1111/nyas.12396.

Rowhani, P.; Lobell, D. B.; Linderman, M.; Ramankutty. N. Climate variability and crop production in Tanzania. *Agric. For. Meteorol*. 2011, 151(4), 449–460, doi: 10.1016/j.agrformet.2010.12.002.

Ruelland, E.; Zachowski. A. How plants sense temperature. *Environ. Exp. Bot*. 2010, 69(3), 225-232: https://doi.org/10.1016/j.envexpbot.2010.05.011

Sabagh, A.E.; Hossain, A.; Iqbal, M.A.; Barutçular, C.; Islam, M.S.; Çiğ, F.; Erman, M.; Sytar, O.; Brestic, M.; Wasaya, A.; Jabeen, T. Maize adaptability to heat stress under changing climate. *J. Plant Stress Physiol.* 2020, DOI: http://dx.doi.org/10.5772/intechopen.92396

Shiferaw, B.; Prasanna, B.M.; Hellin, C.; Bänziger. M. Crops that feed the world. Past successes and future challenges to the role played by maize in global food security. *Food Secur*. 2011, 3(3): 307–327. doi: 10.1007/s12571-011-0140-5.

Sibiya, J.; Tongoona, P.; Derera, J; van Rij, N. Genetic analysis and genotype× environment (G× E) for grey leaf spot disease resistance in elite African maize (Zea mays L.) germplasm. *Euphytica*, 2012. *185*, 349-362: doi: 10.1007/s10681-011-0466-2.

Sultan, B.; Gaetani.M. Agriculture in West Africa in the twenty-first century: Climate change and impacts scenarios, and potential for adaptation. *Front. Plant Sci*. 2016, 1–20. doi: 10.3389/fpls.2016.01262.

Tesfaye, K.; Zaidi, P.H.; Gbegbelegbe, S.; Boeber, C.; Rahut, D.B. Climate change impacts and potential benefits of heat-tolerant maize in South Asia. *Theor. Appl. Climatol*. 2017, 130(3–4): 959–970. doi: 10.1007/s00704-016-1931-6.

Thayil, V.M.; Zaidi, P.H.; Seetharam, K.; Das, R.R.; Viswanadh, S.; Ahmed, S.; Miah, M.A.; Koirala, K.B.; Tripathi, M.P.; Arshad, M. at.al. Genotype-by-environment interaction effects under heat stress in tropical maize. *J. Agron*. 2020, 10(12). doi: 10.3390/agronomy10121998.

Tiwari, Y.K.; Yadav. S.K. High Temperature Stress Tolerance in Maize (*Zea mays L*.): Physiological and Molecular Mechanisms. *J. Plant Biol*. 2019, 62(2): 93–102. doi: 10.1007/s12374-018-0350-x.

Van der Velde, M.; Wriedt, G.; Bouraoui, F. Estimating irrigation use and effects on maize yield during the 2003 heatwave in France. *Agric. Ecosyst. Environ.* 2010, 135(1–2): 90–97. doi: 10.1016/j.agee.2009.08.017.

World Food Programme. El-Nino devastates Zimbabwe's harvest, WFP using satelite to mitigate effects of heat stress. 2016, https://www.fao.org/family-farming/detail/fr/c/449757/: Accessed (3/06/2023).

Worku, M.; Makumbi, D.; Beyene, Y.; Das, B.; Mugo, S.; Pixley, K.; Banziger , M.; Owino, F.; Olsen, M.; Asea, G.; Prasanna, B,M. Grain yield performance and flowering synchrony of CIMMYT's tropical maize (*Zea mays L.*) parental inbred lines and single crosses. *Euphytica*, 211(3): 395–409. doi: 10.1007/s10681-016-1758-3.

Zaidi, P.H.; Nguyen, T.; Ha, D.N.; Thaitad, S.; Ahmed, S.; Arshad, M.; Koirala.; Rijal, R.T.; Kuchanur, P.H.; Patil, A.M et al. Stress-resilient maize for climate-vulnerable ecologies in the Asian tropics. *Aust. J. Crop Sci.* 2020, 14(8): 1264–1274. doi: 10.21475/ajcs.20.14.08.p2405.

Zaidi, P.H.; Vinayan, M.T.; Nair, S.K.; Kuchanur, P.H.; Kumar, R.; Singh, S.B.; Tripathi, M.P.; Patil, A.; Ahmed, S.; Hussain, A. et al. Heat-tolerant maize for rainfed hot, dry environments in the lowland tropics: From breeding to improved seed delivery. *Crop J. 2023*, 11(4): 986–1000. doi: 10.1016/j.cj.2023.06.008.

Ziyomo, C.; Bernardo, R. Drought tolerance in maize: Indirect selection through secondary traits versus genomewide selection. *Crop Sci.* 2013, *53*(4), 1269-1275: doi: 10.2135/cropsci2012.11.0651.

CHAPTER 2

Maize breeding for heat stress tolerance: A review.

Abstract

Heat stress is one of the primary abiotic stresses significantly affecting current and future global maize production. In sub Saharan Africa (SSA), heat stress affects smallholder farmers in stress-prone environments who rely on maize for their living. Climate change-driven heat stress results in a physiological water shortage because of the demand-side drought triggered by a rise in vapor-pressure deficit (VPD). The increase in frequency of extreme temperatures and prolonged drought period due to climate change has important implication on food security in SSA and the region is vulnerable to climate change impacts. The use of stress tolerant germplasm has been reported as the most effective way to mitigate the effects of heat stress. However, heat stress breeding efforts in SSA are in the infancy stage. Development of heat tolerant breeding strategy requires understanding of the mechanism of heat stress tolerance, causes and impacts of heat stress, plant growth and developmental processes, as well as genetic diversity for desired traits. This review aims to provide a synopsis of the genetic basis of heat stress in maize, the response of maize crop to heat stress, breeding strategies for improving heat tolerance in maize, and the progress made in breeding for heat stress in maize. Reviewing the available information will help to improve breeding efficiency and selection of appropriate breeding strategies to enhance breeding for heat stress tolerance in maize in SSA.

Keywords: Climate change, heat stress, grain yield, quantitative trait locus, exotic germplasm

2.1 Background

Maize (Zea mays L.) is the third most significant cereal crop in the world after wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) (Sandhu et al. 2007; FAO, 2021). Its demand is rapidly increasing owing to population growth, food preferences, and socio-economic factors (Agrawal et al. 2018). Majority of people in Sub-Saharan Africa (SSA) depend on maize for food, animal feed, industrial raw materials and as a source of income (Agrawal et al. 2018). In SSA, maize is predominantly grown by resource-poor smallholder farmers in marginal areas as a rain-fed crop (Prasanna et al. 2021). The region has been recognized as a hotspot for climate change (Cairns et al. 2013) and has poor adaptive capacity to extreme events (Zaidi et al. 2020). In Zimbabwe, most maize famers are located in heat and drought stress prone areas, but no heat-tolerant variety has been registered in the country (Mukaro et al. 2023).

Heat and drought stress usually occur simultaneously but resistance to each stress is genetically unique (Nelimor et al. 2020). Between 1980 and 2008, variations in temperature have caused a 3.1% decrease in maize production worldwide, and changes in rainfall patterns have caused a 0.7% decline (Lobell et al. 2011). This has resulted in a 3.8% reduction in the total yield of maize (Lobell et al. 2011). Heat stress alone or in combination with drought has caused considerable grain yield losses in SSA, and temperature is predicted to rise in SSA (Nelimor et al. 2020). Heat stress has become an essential trait in most breeding programs in SSA and a top priority in the International Institute of Tropical Agriculture – Maize Improvement programme (IITA-MIP) (Badu-Apraku and Fakorede, 2017) and the International Maize and Wheat Improvement Center- Global Maize Program (CIMMYT- GMP) (Cairns and Prasanna, 2018).

Heat stress is generally defined as a rise in temperature beyond threshold level that cause irreversible damage to plant growth and development over a prolonged period of time (Wahid et al. 2007; Teixeira et al. 2013; Govindaraj et al. 2018). Compared to drought stress, heat stress has caused a larger drop in maize yields (Rowhani et al. 2011; Prasanna et al. 2021) because plants under heat stress experience physiological drought, which cause increases vapor pressure deficit (VPD) and necessitates higher water demand for evapotranspiration (Zaidi et al. 2023). According to Lobell et al. (2011) a 20% drop in rainfall has less effect on maize grain yield than a 2°C rise in temperature. Several models have predicted rise in average global temperature of between 10°C to 37°C, with yield decrease greater than 7.4% for every 1°C increase in mean global temperature (Ni et al. 2018; Ayenan et al. 2019). Similarly, a 3–4°C increase in temperature was predicted to cause a 15–35 % grain yield reduction across Africa and Asia and a 25–35% grain yield reduction in the Middle East (Govindaraj et al. 2018). Maize grain yield loss between 7% and 40% due to heat stress was reported by Valdés-López et al. (2016), Ni et al. (2018), and Meseka et al. (2018) and similarly, Khodarahmpour et al. (2011) reported a 70% grain yield reduction. In southern Africa, a study of more than 20,000 past maize yield trials revealed that maize productivity declined linearly with each degree day beyond 30°C (Lobell et al. 2011). Thompson (1966) reported a 10% grain yield loss when temperatures rose from 22 to 28°C during the grain filling stage in the United States Corn Belt, while a 42% grain yield decline was recorded when mean daily temperatures rose by 6°C (Badu-Apraku et al. 1983).

Elevated nighttime temperatures increase the rate of respiration in maize plants. This heightened nocturnal respiration results in a wasteful loss of photosynthetically-produced sugars, thereby reducing the net accumulation of dry matter in the plants. Past research has revealed that above-average nighttime temperatures during the grain filling stage in maize can significantly reduce yields by decreasing both kernel number and individual kernel weight (Lutt et al. 2016). As nighttime temperatures rise, the rate of plant respiration increases rapidly. With high overnight temperatures, a greater proportion of the sugars generated through daytime photosynthesis are consumed in respiration, leaving less available to support the development and filling of the kernels. Further, high nighttime temperatures accelerate the accumulation of heat units or growing degree days (GDDs), which can trigger earlier crop maturation. In contrast, cooler nighttime conditions slow GDD accumulation, prolonging the grain filling period and facilitating greater dry matter deposition and improved grain yields (Lutt et al. 2016).

Despite the detrimental effects of heat stress on maize crop and the reports on significant yield loss, breeding efforts for heat stress are still in the infancy stage (Cairns et al. 2013; Alam et al. 2017). Hence, heat stress has become a prominent area of concern for plant scientists (Tiwari and Yadav, 2019). The analysis that was done in SSA on maize germplasm from CIMMYT and IITA breeding programmes under high temperature conditions revealed that germplasm from Eastern and Southern Africa (ESA) is susceptible to heat stress (Prasanna et al. 2021). These results prove that heat stress should be a part of the ESA's maize breeding objectives.

2.2 Physiological response of maize to heat stress

Heat stress, like any other abiotic stress, it triggers a complex of morphological, physiological, anatomical, and molecular changes that affect maize growth and development (Wahid et al. 2007; Badu-Apraku and Fakorede, 2017). These effects vary depending on the severity of the stress, duration, rate at which the temperature rises, and the growth stage at which the crop is subjected to stress (Ruelland and Zachowski, 2010; Kaushal et al. 2016; Fahad et al. 2017). Heat stress causes rapid accumulation of heat units (Basavaraj and Rane, 2020), leading to fast initiation of growth phases prior to the buildup of sufficient resources (Zinn et al. 2010) and ultimately a decrease in productivity (Aiken, 2009; Cairns et al. 2012, Tao et al. 2016).

Heat stress affects several physiological processes, such as photosynthesis, respiration, osmolytes concentration, phytohormone levels, stomatal conductance, and membrane stability (Tiwari and Yadav, 2019). Acute physiological drought due to increased atmospheric moisture demand under heat stress cause substantial VPD (Seetharam et al. 2021; Zaidi et al. 2023). Chlorophyll biosynthesis is inhibited under heat stress (HTS) leading to reduced photosynthates production, and distribution (Hasanuzzaman et al. 2013), and eventually reduced biomass and final grain yield (Chaudhary et al. 2020). A 50–60% decrease in net photosynthetic rate due to HTS was reported by Ben-Asher et al. (2008). The source-sink relationship refers to the dynamic balance between the production of photosynthates (the "source") and their utilization or storage (the "sink"). This relationship is directly affected by heat stress, and this reduces the amount of photo-assimilates available to the growing seeds (Chaudhary et al. 2020).

Heat stress significantly impacts the water-related processes in maize plants. It increases the rate of water evaporation from the soil surface, leading to faster depletion of soil moisture. This reduced soil water availability can limit the amount of water accessible to the plant's roots. Additionally, heat stress elevates the rate of transpiration from the plant's leaves (Yang et al. 2023). Under high temperatures, the plant's stomata (pores) tend to open wider to facilitate cooling through evaporative heat loss, resulting in greater water loss through transpiration. Excessive transpiration can cause wilting and decrease the water available for other critical plant functions. Furthermore, heat stress augments both evaporation and transpiration, resulting in a rise in the overall evapotranspiration rate. This heightened evapotranspiration leads to increased water demands and more rapid depletion of soil moisture, potentially subjecting the maize plants to drought stress (Tiwari and Yadav, 2019).

Heat stress causes electrolyte leakage and decreased the plasma, organelle and vacuolar membranes stability resulting in membrane damage (Wahid et al. 2007). Membrane permeability increases under heat stress, causing electrolytes release, loss of grana stacking and swelling (Wang et al. 2018). Grana are stacked, disc-shaped structures called thylakoids found within the chloroplasts of plant cells. The stacking of the thylakoid membranes into grana structures is an important adaptation that enhances the efficiency of light absorption, energy conversion, and the overall photosynthetic process in plant cells. Additionally, the stresses reduce photosynthetic carbon assimilation through temperature-induced reduction of pyruvate carboxylase enol phosphate (PEPC) and Rubisco activity. The activity of photosystem II (PSII) is also reduced and its repair is prevented by the production of reactive oxygen species (Tao et al. 2016). Heat stress changes the plant hydraulic conductance and affects water balance, membrane stability of leaves (Kaushal et al. 2016) and the plant root system, which supplies water and nutrients to other plant organs (Valdés-López et al. 2016). The efficiency of cell enzymatic operations, stability of plasma membranes, cytoskeleton organization, and metabolic imbalance are largely affected by heat stress (Hussain et al. 2019).

At molecular level, heat stress causes oxidative stress, production of stress proteins, and altered genomic methylation (Kandel et al. 2018; Hussain et al. 2019). High temperatures cause an increase in membrane fluidity due to protein denaturation and the rise in amounts of unsaturated fatty acid (Wahid et al. 2007). In heat sensitive maize cultivars, salicylic acid decreases, and abscisic acid and indole acetic acid increase (Dinler et al. 2014) an indication of suboptimal hormonal response to heat stress which can impair the plant's ability to mount an effective defense, leading to reduced tolerance, stunted growth, and ultimately, lower yields. When maize crops are exposed to heat stress, they accumulate low-molecular-weight organic molecules such as proline, sugars and sugar alcohols, osmolytes, ammonium, and sulphonium compounds (Wahid et al. 2007). The build-up of endogenous butane and osmolytes in maize enhances heat tolerance but causes multiple mineral deficiencies (P and Fe) in roots and shoots, which in turn increases ethylene production (Peverelli and Rogers, 2013). Heat stress adversely affects the plant's ability to absorb adequate- concentration of minerals and nutrients needed for normal metabolic and physiological functions (Fahad et al. 2017).

Effects of HTS on maize crop at different phases of growth have been thoroughly reviewed (Teng et al. 2022; Zaidi et al. 2023). At germination stage, HTS cause seed germination failure, poor plant emergence, poor seedling vigor, and reduced radicle and plumule growth of geminating seedlings

(Hasanuzzaman et al. 2013). Prolonged HTS and sudden heat shock significantly reduce membrane stability, and chlorophyll concentration in maize seedlings (Yadav et al. 2018). Two weeks prior to flowering, HTS lowers chlorophyll concentration and speeds up leaf senescence (Meseka et al. 2018). At the onset of flowering the stress prolongs the anthesis-silking interval (ASI) (Longmei et al. 2021), resulting in poor synchronization and eventually reproductive failure (Prasanna et al. 2021). Bassu et al. (2014) predicted a 45% loss in global maize yield by 2080 due to heat stress at flowering stage. During seed development, heat stress was found to reduce maize quality parameters such as starch, protein, and oil content (Fahad et al. 2017).

Reproductive stage is the most susceptible stage to HTS (Hasanuzzaman et al. 2013; Fahad et al. 2017). At this stage, HTS prevents meiosis, impedes pollen germination and elongation of pollen tube, decrease ovule viability and size (Herrero and Johnson, 1980), interferes with fertilization processes, affects embryo fertilization, and slows down endosperm growth (Chaudhary et al. 2020). Thompson, (1966) and Edreira et al. (2014) reported 10% and 23% yield loss respectively at the grain-filling stage in maize. Similarly, Smith (1996) reported maize yield drop of 101 kg/ha/day when the temperature goes beyond 35^{o}C at the reproductive stage. Considerable yield loss due to sterility, poor ear development, ear abortion, and premature black layer formation was reported in maize under heat stress (Tiwari and Yadav, 2019; Chaudhary et al. 2020; Basavaraj and Rane, 2020; Longmei et al. 2021). Similarly, kernel weight reduction of 57-80% was observed in maize under heat stress (Alam et al. 2017).

Leaf firing, leaf senescence, tassel blasting, premature lodging, leaf rolling, reduced plant height, and reduced internode length are some of the morphological effects of heat stress on maize (Chen et al. 2012; Alam et al. 2017; Meseka et al. 2018). Yield losses from these effects was estimated to be greater than 45 percent (Bassu et al. 2014; Sultan and Gaetani, 2016; Zaidi et al. 2023).

2.3 Heat stress mitigation strategies in maize

Different strategies have been employed to alleviate effects of HTS on maize crop. These include management practices, such as proper plant nutrition, irrigation, suitable planting dates, use of early maturing varieties, crop rotation, mulching, sub-soiling, ridging, foliar application of plant hormones, and seed treatment prior to planting (Wahid et al. 2007; Lipiec et al. 2013; Sehgal et al. 2018). Additionally, external protectants such as antioxidants (ascorbic acid and glutathione), osmoprotectants (glycine betaine, proline) plant growth regulators (Salicylic acid, abscisic acid), nutrient supplements (calcium, boron) and organic amendments (humic acids, biochar) have been successfully used to minimize heat stress in maize crop (Waraich et al. 2012). Improvement in water management has been used positively to alleviate heat stress in plants as they transpire to maintain stable leaf temperature (Khan et al. 2019).

Application of organic mulch to the soil surface lowers heat conductivity and reduces soil temperature (Khan et al. 2019). However, this may promote termite attack and reduce plant stand. Plants grown on ridges withstand HTS because tillage permits heat to escape from the soil to the atmosphere (Lipiec et al. 2013, Tao et al. 2016). Planting early maturing cultivars is an approach that has been used to manage the stress in maize with the assumption that the plants escape HTS (Khan et al. 2019). However, this practice is associated with a yield reduction penalty. Adjustment of planting dates (advancing or delaying) is a farmer-friendly and biologically viable strategy which works with the presumption that heat-sensitive stages like flowering and reproductive do not coincide with HTS in the season (Driedonks et al. 2016).

Effects of HTS on maize plants have also been reduced by soil nutrient management (El-Sappa et al. 2022). The use of macronutrients (K, Ca) and micronutrients (B, Se, and Mn), improve membrane stability, maintain turgor pressure (Tao and Zhang, 2010), adjust stomatal function, and trigger physiological and metabolic processes that help plant tissues retain high water potential and enhance heat tolerance (Waraich et al. 2012; El-Sappa et al. 2022). The use of nitrogenous fertilizer helps to exploit absorbed light and carbon absorption, which enhances heat tolerance (Meena et al. 2020). Application of sulfur-based fertilizers to the soil promotes plant growth and development, enhances uptake of other nutrients, and improves plant tolerance to HTS (Mobin et al. 2017). Despite the efficacy of the approaches mentioned above, none of them have been able to totally mitigate HTS effects on maize (Thayil et al. 2020). Some authors have reported the use of heat tolerant varieties as the most sustainable and economic approach to mitigate the negative effects of HTS in maize (Tiwari and Yadav, 2019; Zaidi et al. 2020; Prasanna et al. 2021; El-Sappa et al. 2022; Zaidi et al. 2023).

2.4 Heat stress response mechanism in maize

Plants have inherent mechanisms to manage their normal metabolism (Wang et al. 2004), maintaining their growth and development along with economic yield under heat stress conditions (Rai et al. 2018). The expression of heat stress response mechanisms occurs at the morphological, physiological, biochemical, and phenological levels. The mechanisms are generally classified as stress escape, stress avoidance, and stress tolerance (Kandel et al. 2018, Khan et al. 2019). Plants can show more than one of the three mechanisms at the same time. Understanding these mechanisms is essential to improve heat stress tolerance in maize.

2.4.1 Heat stress avoidance

Heat stress avoidance is a mechanism that allows plants to survive under heat stress while continuing with their normal growth cycles (Hasanuzzaman et al. 2013; Lin et al. 2017). Mechanisms such as leaf rolling, transpirational cooling, stomatal conductance, membrane stability, stay green, root architecture, stomatal closure, senescence, change of leaf orientation, and leaf thickness have been reported by several authors (Wahid et al. 2007; Lin et al. 2017; Khan et al. 2019). At cellular level, the stress avoidance mechanism includes ion transporters, LEA proteins, signaling cascades, osmolytes, changes in the lipid composition of the cell membrane, antioxidant defense, and transcriptional regulation (Rodríguez et al. 2005; Tiwari and Yadav, 2019).

Leaf rolling reduces leaf surface area, interferes with radiation interception, and decreases transpiration (Wahid et al. 2007). The rate of transpiration increases as canopy temperatures rise due to vaporization and VPD (Govindaraj et al. 2018). Under stress conditions, plants maintain high water status by reducing leaf area, closing stomata, and senescing older leaves (Khan et al. 2019). Due to their capacity to decrease direct sunlight exposure and evaporation rate, maize genotypes with high leaf wax, lower leaf angle, compact tassel, and lower cob angle are more suitable for high-temperature stress conditions(Tiwari and Yadav, 2019). Waxy leaves keep tissue water potential high (Ludlow and Muchow, 1990) increase water-use efficiency, provide a cooling effect, and lowers the rate of leaf senescence (Richards et al. 1986). Stay green post-anthesis maintains carbon assimilation and aids in grain filling because of its relationship with photosynthetic capacity and mobilization of stem reserves to grains (Agrawal et al. 2018).

2.4.2 Heat stress escape

Heat stress escape mechanism enables plants to complete their life cycle at a much-accelerated rate before heat stress sets in (Khan et al. 2019, Basavaraj and Rane, 2020) while maintaining their grain yield potential (Rai et al. 2018; Govindaraj et al. 2018; Janni et al. 2020). The escape mechanism allows sensitive physiological processes to occur when conditions are favorable. Plants can escape HTS by displaying phenotypic plasticity, which shortens the grain-filling period (Tian et al. 2020). Early flowering and maturity are associated with shorter vegetative phases, high metabolic rates, and high water usage efficiency (Shavrukov et al. 2017) but the net duration of photosynthesis and carbon assimilation is shortened (Kaushal et al. 2016). Under heat stress, plants reduce water loss and increase water uptake as an escape mechanism (Rai et al. 2018). This mechanism has been used by several researchers to improve grain yield under high-temperature environments (Wahid et al. 2007). Given the frequency of terminal heat-stress events in SSA, breeding for early maturity can be a promising strategy. In Zimbabwe, Seed Co Ltd has developed several ultra-early (300 series), very early (400 series) and early maturing (500 series) varieties which can be used by maize producers in heat and drought prone areas (Chikwati, 2022).

2.4.3 Heat stress tolerance

Heat stress tolerance is defined as the ability of a plant to survive the detrimental effects of high temperature and produce economic yields that are comparable to or close to those of optimal conditions under HTS conditions (Wahid et al. 2007; Khan et al. 2019). Plants have stress-responsive mechanism that serves to preserve and restore damaged proteins and membranes under heat stress (Wang et al. 2004). Heat shock proteins (HSPs) confer heat tolerance by maintaining plant functions, efficiently scavenging reactive oxygen species, and stabilizing the structure and function of protein and enzymes (Yadav et al. 2015). Heat-tolerant crops restore homeostasis, protein protection, and damaged systems so that they can cope with heat stress conditions for survival, growth, and development (Kandel et al. 2018). Heat tolerance in maize is also attributed to factors such as lower leaf temperature, higher photosynthetic rate, cell membrane stability, and maximal quantum yield PSII photochemistry, (Yadav et al. 2015).

Transpiration is one of the most typical heat tolerant mechanisms used by plants to put up with heat stress. However, Agrawal et al. (2018) reported that on well-hydrated soils, grain yield loss of at least 40% was observed when plants were exposed to temperatures exceeding 38^{o}C during the flowering stage. This shows that the ability to tolerate HTS by cooling has its own set of challenges. Osmotic adjustment is yet another physiological process that helps maize tolerate HTS (Kandel et al. 2018). The osmotic pressure of the cytoplasm is raised by active solute accumulation, which preserves the water balance of the cell and lessens water loss and the negative effects of heat stress (Kandel et al. 2018). Heat-tolerant characteristics preserve tissue hydrostatic pressure by metabolic and cellular changes, mostly through osmotic adjustments (Khan et al. 2015). Another important physiological process that improves heat tolerance in maize is the maintenance of an ideal photosynthetic rate (Tiwari and Yadav, 2019).

In maize, several antioxidant enzymes, including Superoxide dismutase, catalase, ascorbate peroxidase, and peroxidase are associated with HTS (Gill and Tuteja, 2010). Yadav et al. (2015) reported that the ascorbate-glutathione cycle enhances heat tolerance on maize during the reproductive stage. Heat shock factors (HSFs) and heat shock proteins (HSPs) protect cells from the harmful effects of heat stress (Nadeem et al. 2018). Under HTS, heat-tolerant plants accumulate osmolytes such as sugar molecules, proline, glycine betane, and trehalose that make plants more resilient to high-temperature stress (Yadav et al. 2015; Tiwari and Yadav, 2019). Maize cultivars with long and erect top leaves shield the tassel from direct sunlight and hence confer HTS tolerance.

2.5 Genetic basis of heat stress tolerance in maize

Heat stress is a complex quantitative characteristic whose inheritance is influenced by several genes, each contributing a small effect to the overall phenotypic expression (Kandel et al. 2018; Thayil et al. 2020). The contribution of several genetic and environmental factors makes selection process complicated. Therefore, understanding the nature and extent of the gene action influencing the inheritance of heat-tolerant traits is required to make the optimum parental choices and breeding decisions (Kandel et al. 2018; Zaidi et al. 2023). So far very few studies have been published on the inheritance of heat tolerant traits in maize and the reports have been contradictory (Naveed et al. 2016; Jodage et al. 2018; Zhao et al. 2019; Zaidi et al. 2023).

Additive, non-additive, and epistatic gene actions regulate heat-tolerant traits, with one of these effects predominating (Kandel et al. 2017; Ayenan et al. 2019). Functional genomics, transcript profiling, and combining ability studies are some of the approaches used to identify the gene action responsible for heat tolerance in maize (Naveed et al. 2016; Zhao et al. 2019; Kandel et al. 2017). According to Uddin et al. (2008), dominance gene action primarily regulates the inheritance of majority of heat tolerant traits in maize. Prior research has demonstrated that, under HTS conditions, both additive and non-additive gene action affect maize GYD and yield-related traits (Tassawar et al. 2012; Dinesh et al. 2016; Jodage et al. 2017; Longmei et al. 2020; Mukaro et al. 2023). The inheritance of plant height, chlorophyll concentration, grain yield, and biomass per plant was reported to be controlled by both additive and non-additive gene effects under heat stress conditions (Muraya et al. 2006; Jodage et al. 2017; Patil et al. 2022).

Several authors reported non-additive gene action as primarily responsible for the inheritance of days to 50% flowering, plant height, ear height, and grain yield under HTS conditions (Tassawar et al. 2007; Jodage et al. 2017; Alam et al. 2017; Zaidi et al. 2023). Similarly, inheritance for ASI, tassel blasting, leaf firing, ear girth, leaf rolling, leaf senescence, silk receptivity, shelling percentage, number of ears per plant, days to maturity, 100-grain weight, grain yield and yield attributing traits are primarily controlled by non-additive gene action (Tassawar et al. 2012; Khodarahmpour et al. 2011; Alam et al. 2017; Jodage et al. 2017; Kandel et al. 2018). The inheritance of seed vigor, germination percentage, plant vigor, plant growth rate, relative water content, pollen size and viability, leaf senescence, and days to maturity are largely controlled by non-additive gene action under heat stress conditions (Tassawar et al. 2007; Jodage et al. 2017). Khodarahmpour et al (2011) and Jodage et al (2018) reported that the inheritance of cell membrane thermo-stability, stomatal conductance, chlorophyll concentration, transpiration rate, turgor potential, growing degree days to 50% tasselling and leaf rolling were governed by non-additive gene effect under heat stress conditions. Genetic analysis of heat stress tolerance-associated traits revealed the predominance of non-additive gene action in the inheritance pattern of tassel blast and leaf firing (Jodage et al. 2017).

Additive gene action was reported on the inheritance of chlorophyll concentration, plant height, leaf curling, and plant vigor (Tuberosa and Salvi, 2006). Khodarahmpour et al. (2011) conducted a diallel study using eight maize inbred lines under HTS and optimal conditions and found that GYD had high general combining ability to specific combining ability ratio showing the relative importance of additive genetic inheritance under heat stress conditions. The growing degree days to 50% maturity is governed by additive genes effect at high and normal temperature conditions (Dinesh et al. 2016). Generation mean analysis revealed substantial broad and narrow sense heritability (more than 60%) for heat-adaptive traits like leaf temperature and cell membrane thermo-stability, demonstrating the importance of additive gene action in their inheritance (Naveed et al. 2016). Similarly, spring maize showed significant combining ability impacts for membrane thermo-stability under heat stress conditions (Dinesh et al. 2016). Epistatic interactions for leaf firing, shelling percentage, and membrane thermo-stability were reported under heat stress (Fasahat et al. 2016). Available information supporting inheritance of HTS tolerance traits in maize is contradictory. Therefore, there is need to conduct further research to elucidate the nature of gene activity regulating the expression of heat tolerant traits under heat stress conditions.

Early generation selection is effective for traits controlled by additive gene action whereas selection at later stage would be beneficial for traits controlled by non-additive gene action (Dinesh et al. 2016). Under HTS conditions, traits controlled by non-additive gene action can be improved by hybrid breeding and the frequency of favorable heat-tolerant genes in maize populations can be increased by population improvement programs (Kandel and Shrestha, 2018). The use of additive gene effects in breeding programs is more effective and less expensive (Fasahat, 2016).

2.7 Exploiting exotic germplasm for heat stress tolerance in maize

Exotic germplasm is a valuable genetic resource for the development of improved cultivars (Michelini and Hallauer, 1993). Several authors have emphasized the importance of incorporating exotic genes into adapted germplasm to increase heterotic vigor (Iglesias, 1989), broaden the genetic base of elite germplasm, increase allelic frequency, and provide distinct productivity-enhancing alleles lacking in elite crop gene pools (Beck et al. 1991; Betrán et al. 2006; Holland, 2004). However, in many breeding programmes, breeders use a small portion of exotic germplasm (Dinash et al. 2016; Jodage et al. 2017; Zaidi et al. 2023) fearing the presence of undesirable photoperiod reactions that mask desirable attributes, the length of time required to extract useful genes (Dwivedi et al. 2016), and inability to break links between favorable and unfavorable genes (Iglesias, 1989, Ndoro, 2019). Lack of data to guide the selection of good germplasm, and the presence of undesirable traits that make germplasm difficult to evaluate, maintain, and use are some of the reasons given for the limited use of exotic germplasm (Iglesias, 1989).

The use of exotic germplasm to improve heat stress tolerance in maize is steadily growing because of the mega-environment (areas with broadly similar environmental characteristics with respect to maize production) concept that was developed by CIMMYT (Dwivedi et al. 2016; Prasanna et al. 2021). In Zimbabwe, exotic heat tolerant donor lines obtained from CIMMYT-India were used by CIMMYT-Zimbabwe to incorporate heat tolerance genes into locally adapted germplasm (Mukaro et al. 2023). The exotic heat tolerant donor lines showed superior performance under optimum and heat stress conditions in Zimbabwe and are now used in heat stress breeding in Zimbabwe (Mukaro et al. 2023). Some national research institutions in SSA such as Kenya, Mozambique, Nigeria, South Africa, Tanzania, Zambia and Zimbabwe have reported similar initiatives (Nelimor et al. 2019; Thayil et al. 2020; Badu-apraku et al. 2023).

2.8 Genotype X Environment interaction

Genotype by environment interaction (GEI) has been defined as inability of a genotype to perform consistently in different environments and locations (Setimela et al. 2007). The presence of GEI is important to plant breeders as it complicates the selection and recommendation of best cultivars from multi-locational evaluations (Nzuve et al. 2013; Matova et al. 2022). Crossover interaction refers to a situation where the relative performance of different genotypes changes across different environmental conditions. It is the most important in crop improvement because it results in change in the magnitude and rank of genotypes (Bänziger and Cooper, 2001; Gasura et al. 2015).

Studies of GEI in maize under stress and optimal conditions were conducted and several authors reported large and significant GEI under stress environments (Setimela et al. 2007; Sibiya et al. 2012; Ndhlela et al. 2014; Gasura et al. 2015; Makumbi et al. 2015; Shaibu et al. 2016; Sserumaga et al. 2016). Useful strategies used to address GEI include conducting stability analysis to identify high yielding and stable cultivars and breeding for target environments (Matova et al. 2022). Several statistical methods have been developed to analyse the GEI data and these include analysis of variance (least squares, restricted maximum likelihood), regression, variance components, cluster and factor analyses, the additive main effects and multiplicative interaction model Makumbi et al. (2015); Sserumaga et al. (2016) and the genotype and genotype by environment (GGE) biplot analysis (Yan and Tinker, 2006). Using factorial regression, relative humidity and VPD were identified as the two most significant environmental factors that influence the significant GEI observed on GYD under HTS conditions (Thayil et al. 2020). The GEI for heat stress tolerance under field environment could be relatively explained by the performance under controlled environment (Telfer et al. 2018).

2.9 Breeding for heat tolerance in maize

Heat stress tolerance in maize has been improved using a wide range of conventional and non-conventional strategies. These strategies are not mutually exclusive, but rather complementary in most breeding systems (Ribaut et al. 2010). Breeding methods such as introductions, hybridization, doubled haploid (DH), and modern breeding techniques like quantitative trait locus (QTL) mapping, marker-assisted selection (MAS) (Gupta et al. 2017) and Genomic selection (GS) has been used in tandem to accelerate genetic gains in maize abiotic stress breeding (Shikha et al. 2017; Corazza et al. 2020). Multi-locational evaluation of maize genotypes and index selection for heat stress-adaptive secondary traits under stressed and optimal conditions, and correlated traits are other approaches used for HTS breeding in maize (Zaidi et al. 2020).

2.9.1 Conventional breeding

Since the twentieth century, conventional breeding methodologies have successfully resulted in the development of maize genotypes with improved grain yield, and stress tolerance through crossing and selection over years (Liu and Qin, 2021). Conventional breeding efforts for heat-tolerant maize inbred lines and hybrids primarily use two different strategies which are screening of the available germplasm as well as planned and targeted breeding for the development of heat-tolerant maize inbred lines and hybrids (Mallikarjuna et al. 2020). However, the rate of productivity growth using the conventional method is insufficient to meet the future demand for maize due to human population expansion and growing climate variability (Lamaoui et al. 2018; Prasanna et al. 2021).

2.9.1.1 Multi-locational evaluation under heat stress

Evaluation of local germplasm under HTS was done in different regions including Asia, Africa and Latin America and heat tolerant hybrids with excellent GYD were identified (Chen et al. 2012; Mallikarjuna et al. 2020; Mukaro et al. (unpublished). In Pakistan the hybrids identified includes FH-988, FH-992, FH-1046, and YH-1898 (Yousaf et al. 2018; Mallikarjuna et al. 2020). Furthermore, VL05728 and VL05799 lines from CIMMYT-Asia showed better reproductive performance after being assessed alongside 75 distinct inbred lines under HTS (Alam et al. 2017). In Zimbabwe, local hybrids PGS3 from Progene seeds, SC555 from Seed-co and SYNG1 from Syngenta have shown good yields under HTS (Mukaro et al Unpublished). In Texas, USA, several heat tolerant lines (B76, TX205, C273, BR1, B105C, C32B, S1W, and C2A554-4) were identified at reproductive stage from the HTS evaluations conducted under field and greenhouse conditions (Chen et al. 2012).

Multi-locational evaluation of maize under high temperatures during HTS sensitive developmental stages has identified extensive genetic diversity for heat tolerance in subtropical and tropical maize breeding programmes (Prasanna et al. 2021). Although selection for HTS tolerance in maize has been successful, very little genetic gains have been achieved due to the extensive screening procedure used to identify appropriate germplasm to start a HTS breeding programme (Badu-apraku et al. 2023). Selection was successful when the screening environments closely mimic the target environments and limited genetic gains were obtained when extensive screening required to capture the complexity is time-consuming, labor-intensive, and expensive, limiting the scale and scope of the breeding program. Additionally, some challenges faced by maize scientist which has affected progress in selecting for heat stress tolerance includes the complexity of the traits and the quantitative inheritance of the genes linked to heat stress tolerance (Zaidi et al. 2023).

2.9.1.2 Doubled haploid technology

The doubled haploid is a cutting-edge technology that has improved breeding efficiency and accelerates the introduction of new traits into elite maize breeding lines (Gedil and Menkir, 2019). The technology's ability to shorten the breeding cycle by 6-7 generations makes it a useful tool for plant breeding (Sleper and Bernardo, 2016; Chaikam et al. 2019). Commercial maize breeding programmes in North America, Europe, and most recently China have been using the DH approach over the last ten to fifteen years (Andorf et al. 2019; Trentin et al. 2020). Currently, DH technology is in use by more than 80% of breeding programmes in SSA, including national breeding programmes with the support from CIMMYT through the Accelerating Genetic Gains (AGG) project (Prasanna et al. 2021). The DH technology has been optimized for tropical and subtropical maize growing conditions through the collaboration between the University of Hohenheim, Germany, and the CIMMYT International Maize Programme (Chaikam and Prasanna 2020).

Since 2007, CIMMYT Maize Program has been phenotyping DH populations for heat stress tolerance in various locations throughout the world (Trachsel et al. 2019) and this has demonstrated usefulness in developing superior maize DH lines for utilization in breeding pipelines across SSA, Latin America, and Asia (Chaikam et al. 2019). More than 80 multiple stress tolerant improved hybrids developed using the DH technology were released between 2012 and 2017 by different national breeding programmes including Kenya, Uganda, Tanzania, Mexico, and South Africa (Beyene et al. 2017). These hybrids perform better under optimal, drought, heat, and low nitrogen stress conditions (Beyene et al. 2017). The application of DH technology for heat stress tolerance breeding in SSA is encouraging because of its capacity to shorten the breeding cycles, enhance genetic gain under heat stress and facilitate trait introgression (Chaikam et al. 2019; Beyene, 2022). However, the cost of DH technology implementation could be problematic for SSA small breeding programmes with limited funding.

2.10 Modern breeding approaches for heat stress tolerance in maize

Molecular breeding techniques have been widely and successfully used in the private sector, but there is now an increased emphasis on the application of these approaches in the public sector (Eathington et al. 2007, Dwivedi et al. 2007). The techniques such as quantitative trait loci (QTL) mapping, genome sequencing and genome-wide association study (GWAS) are considered efficient for accelerating genetic gains in maize (Shikha et al. 2017; Prasanna et al. 2021). However, these have not been widely employed in breeding maize for heat stress tolerance (Badu-apraku et al. 2023).

2.10.1 Marker-assisted breeding

Marker assisted selection (MAS) is an efficient breeding methodology extensively used for traits regulated by major genes (Tayade et al. 2018). This approach was effectively used in the development of improved maize genotypes for stress-prone environments (Beyene et al. 2016). Quantitative trait loci are classified based on their stability effect across environments. Those that are detected across environments are called constitutive and those that are detected under specific environmental conditions are adaptive QTLs (Waqas et al. 2021). Given the severe effects of heat stress on grain yield and yield related traits in maize production, the use of constitutive QTL associated with heat stress tolerant traits under heat stress condition would improve maize yield potential.

The QTL mapping approach was used to understand the genetic association between different stress tolerant traits in maize (Collins et al. 2008). However, the marker-trait association of heat tolerant-related traits has received very little attention (Frey et al. 2016). In maize, restriction fragment length polymorphism (RFLP) markers were initially used to map the heat-tolerant QTLs for yield-attributing traits, cellular membrane stability, quantitative expression of HSP, and radicle growth (Frova and Sari-

Gorla, 1994; Frova et al. 1998). Frey et al. (2016) conducted QTL mapping for heat tolerant traits and identified 5 and 6 QTLs for pollen germination and, pollen tube growth respectively. Additionally, QTLs for heat-tolerant traits such as time to flowering, ASI, leaf firing, and tassel blasting were identified (Frey et al. 2016).

In a mapping population for heat-sensitive index, traits such as leaf length, plant height, leaf greenness, and leaf growth rate, 6 QTLs explaining 7% and 9% of phenotypic variation were discovered (Inghelandt et al. 2019). According to McNellie et al (2018), the bi-parental mapping population formed from B73 × NC350 and B73 x CML103 revealed 12, 8, and 1 QTL for leaf firing, leaf blotching, and tassel blasting, respectively. The study has also identified a significant phenotypic variation of 26.2% on chromosome 3 as the primary QTL for heat-induced plant death. Gao et. al. (2019) used a bi-parental association mapping to identify the QTL for heat tolerance for seed set and discovered four QTLs using linkage mapping, and 17 genes associated with 42 SNPs through GWAS. Although these studies were useful in locating the QTLs, some of them had drawbacks, such as the use of small population sizes, which has an impact on the resolutions of the identified QTLs, low heat stress intensity, instability of some of the identified QTLs across environments, and unvalidated QTLs in diverse genetic backgrounds and across environments.

2.10.2 Genome-wide association studies

Genome-wide association is one of the advances of association mapping used to identify markers located much closer to the gene of interest (Singh et al. 2020) and an important tool for linking the genotype–phenotype map (Shikha et al. 2017). This approach is important for understanding the entire set of genetic variants in crop cultivars (Sabagh et al. 2020), improvement of complex traits (Shikha et al. 2017), identification of genes with useful variation for complex traits (Ni et al. 2018; Wang et al. 2018), prediction of the breeding value of individuals within a breeding population, and provision of high mapping resolution (Singh et al. 2020). Genome-wide association studies were successfully used in rice *(Oryza sativa* L.), and maize to interpret genomic regions associated with various key abiotic stresses (Yano et al. 2016; Shikha et al. 2021).

Genome wide association was recently used to identify new QTLs for heat tolerance in maize and has revealed numerous genes that contribute to heat tolerance during flowering stage (Lafarge et al. 2017). The study by Yuan et al. (2019) for combined heat and drought tolerance revealed 6 significant haplotype associations for GYD on chromosome 4 (bin 4.02–4.03 and 4.04) and significant hotspots for GYD under HTS on chromosome 8 (bin 8.05 and 8.03). Longmei et al. (2021) found 12 significant SNP associations for GYD on a panel of 662 DH lines with tropical provenance from a HTS tolerance mapping study on subtropical maize. These SNPs were discovered on chromosomes 1, 3, 6, 7, and 10, and they account for around 18% of the phenotypic variation (Longmei et al. 2021). On chromosomes 1, 3, and 7, the research discovered substantial haplotype trait connections under heat stress (Seetharam et al. 2021).

Using GWAS approach, Lamaoui et al. (2018) discovered five haplotype blocks and eight SNP variants for grain yield and related secondary traits from a study of 500 different maize lines under heat stress. A total of 300 tropical and subtropical maize inbred lines were subjected to genome-wide association analysis, and 6, 36, and 55 candidate genes for AD, ASI, and GYD were identified respectively (Yuan at al. 2019). Genome wide association study was conducted on sub-tropical maize and important SNPs and haplotype blocks linked to yield-related traits were revealed (Longmei et al. 2021). Similarly, Seetharam et al. (2021) conducted a GWAS study using 543 tropical maize inbred lines from different genetic backgrounds and identified 269 significantly associated SNPs for GYD. The study also revealed a total of 13 SNPs within the QTL regions of HTS-related secondary traits such as leaf firing (11 SNPs), leaf blotching (2 SNPs) and tassel blast (1 SNP) (Seetharam et al. 2021). In the same way, 16 SNPs were discovered within QTL regions for heat susceptibility index for GYD (15 SNPs) and, leaf firing (5 SNPs) (Seetharam et al. 2021). Gao et al. (2019) also conducted a GWAS study using 261 diverse maize lines using 259,973 SNPs and identified 17 genes linked with 42 SNPs for seed-set under heat stress. The identified genomic regions are important for current and future breeding efforts targeted at enhancing heat stress tolerance in maize cultivars through marker-assisted introgression into elite genetic backgrounds (Seetharam et al. 2021).

2.10.3 Genomic selection

Genomic selection (GS) is a relatively new molecular-based selection technique that utilize genome-wide marker information to estimate all marker effects and to choose individuals with high genomic estimated breeding values (GEBV) (Santantonio et al. 2020). Because of its low cost, quick turnaround, and capacity for higher selection intensity, GS is important in genomic prediction based on early-stage yield testing (Yuan at al. 2019). The method is based on the predicted GEBV of the chosen candidates who have genotypic data but lack phenotypic data (Yuan et al. 2019). Genomic selection combines all the available marker data into a prediction model to predict the GEBV of breeding materials with unknown phenotype for selection (Newell and Jannink, 2014; Yuan et al. 2019). This method can detect both major and minor QTLs distributed throughout the genome and is particularly useful for the improvement of polygenic traits (Shikha et al. 2017). Genomic Selection accounts for more genetic variation for a given trait than marker-assisted selection (Crossa et al. 2017). The availability of high-density markers distributed throughout the genome helps to improve GS accuracy and efficiency (Yuan at al. 2019).

CIMMYT's global maize program has incorporated GS into its breeding pipelines and results of some studies have shown the applicability of the approach in genetic improvement for abiotic stresses such as heat and drought (Crossa et al. 2017; Yuan et al, 2019). In the CIMMYT heat stress-tolerance breeding programme, GS has been used in multi-parent synthetic populations as rapid-cycle genomic selection to improve source populations and to extract high- breeding value lines. Additionally, GS approach has been used in the breeding programmes as multiple bi-parental breeding populations developed using elite heat stress tolerant lines. The selected lines are further advanced through additional cycles of breeding, testing, and evaluation to confirm their heat stress tolerance performance and agronomic potential. The bi-parental lines are integrated into the mainstream breeding programs and used as parents to develop new breeding populations.

Genomic selection study conducted for GYD, AD, and ASI under HTS showed that the marker trait-associated SNPs had genomic prediction accuracies of 0.69, 0.61, and 0.28, respectively (Yuan et al. 2019). The genomic prediction accuracies for GYD and AD were very good and indicate that the genomic selection approach can be effectively applied in the CIMMYT heat stress-tolerance breeding program for these traits. The lower accuracy for ASI suggests further optimization of the genomic selection methodology or the inclusion of additional genetic factors to improve the prediction for this trait. Genomic selection approach reduces the cost of phenotyping, but to some extend it is not optimal because the breeder has to wait three to four months for the plant to mature before collecting the phenotypic data needed to calibrate the prediction model for the un-tested 50% of the material (MacNellie et al. 2018).

2.10.4 Genome editing technology

Genome editing (GE) is a rapidly advancing technology that enables the precise introduction of specified mutations into the plant genome in a highly specific manner (Georges and Ray, 2017). This technology induces changes in the genome and creates new desirable alleles to accelerate the development of new climate-resilient varieties (Waqas et al. 2021). The availability of the genome sequence and efficient transformation techniques are two fundamental prerequisites for introducing editing components into the genome. Zinc finger nuclease (ZFN), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeat (CRISPR/cas9) are the three major genome editing techniques (Georges and Ray, 2017). The ZFN and TALENs were the first GE technologies, but, the advent of CRISPR/Cas9 surpassed them because it is simple, accurate, faster, and less expensive (Nadeem et al. 2018; Singh et al. 2019). When compared with traditional random mutagenesis methods, CRISPR Cas9 technology exhibits superior off-target effects, high precision, and minimal risk, making it an excellent choice for developing high-yielding crops under HTS (Georges and Ray, 2017).

Prime editing is another gene editing approach for altering the genome that can attain different types of editing such as minor indels, transition and transversion mutations and does not require a double-strand break in the DNA (Marzec et al. 2020). This method has a considerable promise for producing improved maize varieties with high yield and resistance to various biotic and abiotic stresses (Marzec et al. 2020). The use of gene editing tools leverage existing knowledge on genes, gene networks, and pathways involved in pollen and ovule sterility, pollen viability, ovule fertility, and photosynthesis (Nadeem et al. 2018). These genes and pathways have been found to be closely linked to heat tolerance, making them valuable targets for improving the performance of crops under heat stress. Potentially editable genes which are important for heat tolerance include those that are involved in biosynthesis pathways and transduction of secondary metabolites as well as osmo protectant (Nadeem et al. 2018).

Genome editing technology has currently been demonstrated as successful for complicated traits like heat and drought tolerance in maize (Shi et al. 2017). Using GE methodologies, it is possible to target significant candidate genes and regulatory elements related to HTS in order to produce heat-tolerant maize genotypes (Nadeem et al. 2018). The Biotechnology Center of Beijing Origin Seed, Limited has successfully used GE technique to modify traits in maize for stress tolerance and such traits include reduced plant height, increased growth period and chloroplast content under HTS (Han, 2021).

2.10.5 Genetic engineering

Genetic engineering is an effective technology for generating novel variation that is not found in naturally occurring populations (Chapman et al. 2012). The genes are introduced into plant cells, using techniques such as Agrobacterium-mediated transformation or biolistic (gene gun) delivery. This approach has enabled the incorporation of isolated individual genes of interest into selected plants to enhance plant adaptability to various stress conditions including HTS (Barnabás et al. 2008; Chapman et al. 2012). Genetic engineering has led to the development of stress tolerant genotypes (Wahid et al. 2007). The crucial aspect that restricts the application of biotechnological tools, like gene transformation and gene expression under heat stress is the incomplete understanding of the mechanisms that increase heat tolerance in plants (Chapman et al. 2012).

Genetic modifications have been done on several crops including wheat, maize, tomato, and rice to improve heat stress tolerance, targeting mostly heat shock proteins (HSPs) and heat stress transcription factors (HSFs) (Casaretto et al. 2016; Trapero-Mozos et al. 2018). So far, genetic engineering for HTS in maize has primarily focused on modifying genes that encode TFs, HSPs, chaperones, organic osmolytes, antioxidants, and plant growth regulators (Ashraf, 2010; Grover et al. 2013). Several attempts have been made to develop heat-tolerant transgenic maize by manipulating known relevant genes isolated from various crop species (Tiwari and Yadav, 2019). Identification and characterization of HSFs as well as their function in controlling HSP genes, have provided the foundation for the development of genetically modified maize with the high HTS tolerance (Ahuja et al. 2010).

Several genes that can be used in development of improved heat tolerant maize transgenic varieties were identified (Tiwari and Yadav, 2019). Maize plants were transformed with rice MYB transcription factor gene OsMYB55 to improve heat and drought stress tolerance through modulating the expression of stress-associated genes (Casaretto et al. 2016). The gene ZmDREB2A from *Arabidopsis* was introduced to maize for the synthesis of HSPs for heat tolerance (Queitsch et al. 2000). Overexpression of the ZmDREB2A in maize induced heat-responsive genes that provide adaptation and survival under heat stress (Queitsch et al. 2000, Casaretto et al. 2016). Similarly, over expression of cytoplasmic heat-stable 6-phosphogluconate dehydrogenase (6PGDH) under heat stress has caused over accumulation of starch in maize endosperm resulting in increased grain size and weight (Trapero-Mozos et al. 2018).

Overexpression of maize WRKY transcription factor ZmWRKY106 has improved heat and drought tolerance in transgenic *Arabidopsis* by regulating expression patterns of genes linked with the ABA-signaling pathway and superoxide dismutase, peroxide dismutase, and catalase (Wang et al. 2018). Similarly, the expression of maize HSP genes, ZmHSP16.9 and ZmHsf06 enhanced the heat and drought stress tolerance in tobacco and Arabidopsis (Li et al. 2015). ZmHsf04-transformed *Arabidopsis* plants exhibited improved heat tolerance by up regulating the expression of natural HSP and stress-related genes (*AtHsp25.3-p, AtHsp18, 2-CI, AtHsp 70B, AtAPX2 and AtGolSI*). Several maize organs have been found to express ZmHsf12 in an upregulated manner in response to heat shock (Li et al. 2015). Overexpression of the heat shock transcription factor HSf05 cloned from maize has improved the heat tolerance level of *Arabidopsis thaliana* (Li et al. 2015). Similar to this, overexpression of ZmHSFA2 in Arabidopsis raised raffinose concentration in leaves by enhancing AtRS5 (Raffinose Synthase) expression and resulted in improved heat stress tolerance in maize (Gao et al. 2019). Despite an increase in the global production of transgenic crops, the benefits, and promising results in addressing abiotic stresses, the general public concerns and complicated legal frameworks are restricting the use of transgenic techniques in breeding (Hemantaranjan, 2014; Lamaoui et al. 2018).

2.11 Physiological traits-based breeding for heat tolerance

Physiological trait-based breeding has gained popularity for selection under stress conditions (Maazou et al. 2016) due to its reliability, effectiveness, and sustainability for abiotic stress breeding (Araus et al. 2008; Alam et al. 2017). The strategy has been successfully used in the identification of stress-tolerant inbred lines and hybrids in breeding of several crops, including maize and wheat (Araus et al. 2008, Cairns et al. 2013; Alam et al. 2017; Cairns and Prasanna, 2018). Direct selection for grain yield under heat stress is associated with low heritability, significant genotype by environment (GEI) interaction, and complexity of major and minor QTLs driving heat stress (Manavalan et al. 2009; Alam et al. 2017; Mcmillen et al. 2022; Zaidi et al. 2023). Indirect selection using physiological traits that are highly heritable and directly correlated with grain yield under stress environments enhance selection response, selection efficiency and breeding progress (Ziyomo and Bernardo, 2013; Maazou et al. 2016; Tesfaye et al. 2018).

Several studies were conducted to identify ideal traits that are useful for indirect selection under heat stress condition. A number of traits have been identified (Edreira et al. 2012; Noor et al. 2019; Longmei et al. 2020; Teng et al. 2022) but not all of them can be used as selection indices in genetic improvement for heat stress. Selection indices refer to mathematical formulas used to combine information on multiple traits into a single value that can be used to rank and select superior genotypes. An ideal trait to use as a selection target should be highly heritable, has high genetic variability, and high genetic correlation with yield under stress conditions. It should be simple, inexpensive, non-destructive, and quick to measure. Additionally, it should be observable before or during flowering to avoid undesirable cross-pollinations (Edmedes et al. 1997; Mhike et al. 2013; Koirala et al. 2021) and it should be associated with stress tolerance rather than avoidance (Zaidi et al. 2023). While selection based on grain yield produce considerably smaller gains under stress, the use of secondary traits has frequently produce better genetic gains (Edmeades et al. 2017).

Tiwari and Yadav (2019) reported net photosynthetic rate, maximum quantum yield PSII photochemistry, leaf surface temperature, transpiration, stomatal conductance, canopy temperature depression, stay green, stem carbohydrate reserves, and chlorophyll content as physiological traits that can be used indirectly for selection under heat stress. Genotypes that exhibit higher net photosynthetic rate, transpiration, stomatal conductance, high chlorophyll content, lower leaf surface temperatures, and less cell membrane damage have tolerance to heat stress (Kumar et al. 2012; Lipiec et al. 2013, Tiwari and Yadav, 2019). Membrane thermostability was reported as a promising trait for estimating heat tolerance while analyzing genetic variability in various crops (Kumar et al. 2012).

Morphological traits such leaf firing, tassel blasting, leaf rolling, leaf senescence, number of ears per plant, percent kernel abortion, leaf angle, leaf position, and the normalized difference vegetation index have been used to distinguish tolerant and susceptible genotypes under heat stress conditions (Gupta et al. 2017, Jodage et al. 2017; Koirala et al. 2021). Maize genotypes that senesce in the lower half of the plant while retaining active photosynthetic machinery in the top half are reported to be heat tolerant (Jodage et al. 2017). Similarly, the number of rows of kernels per cob, grain yield, and 1000-seed weight were other parameters used post-harvest to identify heat stress tolerant maize genotypes (Tiwari and Yadav, 2019). Several mopho-physiological parameters, such as canopy temperature, stomatal conductance, stay green characteristics, waxy leaves, chlorophyll content, leaf firing, and tassel blasting, have been linked by several authors to maize's ability to withstand heat stress (Jodage et al. 2017; Tesfaye et al. 2018; Zaidi et al. 2023; Badu-Apraku et al. 2023).

Among the traits that were identified by different researchers, anthesis silking interval, leaf firing, tassel blasting, and ears per plant have been recognized as the most widely used (Thayil et al. 2020; Jodage et al. 2017).

2.12 Progress made in breeding for heat tolerance

Significant strides have been made towards development of inbred lines and hybrids with increased heat stress tolerance in Asia, Africa and Latin America (Altin et al. 2017; Badu-apraku et al. 2023). The CIMMYT-Mexico maize program screened populations for heat and drought tolerance in the mid-1980s and produced improved populations from Tuxpeño Sequía, La Posta Sequía, DTP yellow, and DTP white through a recurrent selection programme (Edmeades et al. 1997). Promising high heat tolerant lines were identified and used as source material for stress tolerance and generation of bi-parental populations (Edmeades et al. 1997). Drought-tolerant lines obtained from these populations have been employed as donor material for poor nitrogen, heat, and drought stress tolerance breeding in SSA, Asia, and Latin America (Prasanna et al. 2021).

With financial support from the Bill and Melinda Gates Foundation and USAID's Feed-the-Future initiatives, public research institutions, international organizations (CIMMYT and IITA), and private seed companies collaborated on different projects to advance maize heat stress tolerance breeding in Africa, Asia, and Latin America (Prasanna et al. 2021). The organizations identified heat tolerant traits, incorporate heat tolerance genes into local maize germplasm, conducting multi-location trials under heat stress environments, marker-assisted selection, releasing of enhanced heat-tolerant maize varieties, and strengthening breeding programmes (Altin et al. 2017; Prasanna et al. 2021; Badu-apraku et al. 2023). In SSA, the drought tolerant maize for Africa (DTMA), Stress Tolerant Maize for Africa (STMA) and the Accelerated Genetic Gains (AGG) projects have established effective product pipelines with several promising stress-resilient hybrids for release and commercialization (Prasanna et al. 2021).

The International Maize Improvement Consortium (IMIC) Africa and Asia has significantly contributed to breeding for heat stress tolerance in maize by facilitating collaboration and germplasm sharing among breeders. The CIMMYT's Global Maize Program has been actively involved in developing and distributing heat-tolerant maize germplasm to partner institutions through the IMIC initiative. The consortium has facilitated access to a broader genetic pool of maize germplasm with heat tolerance traits to participating institutions (Prasanna et al. 2021). CIMMYT-Asia conducted a genetic trend analysis for the heat stress breeding programme focusing on mean grain yields of field experiments (Zaidi et al. 2023) and the results revealed genetic gains of 6 kg/ ha/ year under high VPD and 118 kg/ ha/ year under low VPD (Prasanna et al. 2021). Some trait donor lines with GCA for heat-stress tolerance have been identified as part of continuous improvement and will be incorporated into the breeding pipeline (Badu-apraku et al. 2023; Zaidi et al. 2023). Asia has received substantial funding from both the public and private sectors targeting heat stress tolerance improvement in maize (Zaidi et al. 2023).

The Heat Tolerant Maize for Asia (HTMA) project established a robust network for heat stress phenotyping in South Asia, well-equipped facilities, and well-trained representatives (Zaidi et al. 2023). The HTMA project generated heat tolerant base germplasm, comprising of pedigree populations, early generation lines, and more than 500 inbred lines which were given to the partners for use in their breeding programmes (Prasanna et al. 2021). CIMMYT-Asia produced heat-tolerant donor lines, including CML579, CML563, CML580, CL-RCY44, G18Seq, P31, CAL181, and CML565, using the source populations HSBC, MPS1, MPS2, MPS3, MPS4, MPS5, and MPS6 (Prasanna et al. 2021). Among the heat stress-tolerant lines identified by the HTMA project, eight lines were registered internationally as CIMMYT Maize Lines (CMLs) and these includesCML562, CML564, CML565, CML578, CML579, CML580, CML613A, and CML614B. The project also licensed over 50 CIMMYT maize hybrids between 2015 and 2020 and sixteen varieties have already been planted on over 20,000 hectares in Africa and Asia (Zaidi et al. 2023). Alam et al. (2017) identified heat-tolerant maize genotypes, VL05728 and VL05799, with improved seed settings due to reproductive success under heat stress. In India and Nepal impact assessment of heat-tolerant maize hybrid seed showed significant gains in using heat tolerant hybrids over non-heat tolerant hybrids under heat stress (Zaidi et al. 2023).

2.13 Conclusion and Future perspectives

The International and national maize research networks have made significant efforts to raise maize yields, and safeguard farmers and consumers amidst increased heat stress frequency and intensity. However, the rising demand for maize consumption and use in the face of climate change calls for continuous scientific efforts towards the development of improved high-yielding heat stress tolerant maize genotypes. It is therefore, important to comprehend the genetic and molecular basis of each of the functional adaptation trait associated with heat stress tolerance in maize (Nadeem et al. 2018). In addition, it is important to understand the underlying genetic basis of maize under heat stress conditions

(Jagtap et al. 2020). Growing understanding of the molecular mechanisms behind maize's ability to tolerate heat stress is probably going to open the door for the development of maize genotypes that produce sufficient economic yields under heat stress conditions.

The time interval between development and deployment of improved germplasm to farmers is long. To accelerate the process in SSA, it is essential to effectively integrate enabling breeding techniques such as field-based precise phenotyping, doubled haploid, genomic selection and prediction (Chaikam et al. 2019). Furthermore, in order to overcome anticipated yield decreases and achieve high yields, breeders' approaches must be reinforced. Inbreeding has historically been used by breeders to develop inbred lines from the source population but due to its lengthy breeding process and genetic side effects, complete homozygosity could never be reached. Therefore, enhancing DH technology can speed up the development of fully homozygous productive inbred lines by shortening the time required to develop inbred lines and to maximize heterosis. To advance competitively and offer farmers climate-smart cultivars, more funding for the public and private sectors is required to support heat stress breeding in maize in SSA.

References

Agrawal, P.K.; Mallikarjuna, M.G.; Gupta, H.S. Genetics and applied genomics of quality protein maize for food and nutritional security. *Biotechnol. Crop Improv*. 2018, 3, 151–178. https://doi.org/10.1007/978-3-319-94746-4.

Ahuja, I.; de Vos, R.C.; Bones, A.M.; Hall, R.D. Plant molecular stress responses face climate change. *Trends Plant Sci.* 2010.15(12), pp.664-674. https://doi.org/10.1016/j.tplants.2010.08.002.

Aiken, R. Climate Change Impacts on Crop Growth in the Central High Plains. 2009. Proce. 21st Annu. Cent. Plains Irrig. Conf. Febr. 24-25: 14–24.

Alam, M.A.; Seetharam, K.; Zaidi, P.H.; Dinesh, A.; Vinayan, M.T.; Nath, U.K. Dissecting heat stress tolerance in tropical maize (*Zea mays L*.). *Field Crops Res.* 2017*, 204*, 110-119: doi: 10.1016/j.fcr.2017.01.006.

Atlin, G.N.; Cairns, J.E.; Das, B. Rapid breeding and varietal replacement are critical to adaption of developing-world cropping systems to climate change. *Glob. Food Sec*. 2017, 12, 31-37. http://dx.doi.org/10.1016/j.gfs.2017.01.008

Andorf, C.; Beavis, W.D.; Hufford, M.; Smith, S.; Suza, W.P.; Wang, K.; Woodhouse, M.; Yu, J.; Lübberstedt, T. Technological advances in maize breeding: past, present and future. *Theor Appl Genet.* 2019, *132*, 817-849: https://doi.org/10.1007/s00122-019-03306-3.

Araus, J.L.; Slafer, G.A.; Royo, C.; Serret, M.D. Breeding for yield potential and stress adaptation in cereals. *CRC. Crit. Rev. Plant Sci.* 2008, *27*(6), 377-412. doi: 10.1080/07352680802467736.

Araus, J.L.; Kefauver, S.C.; Zaman-Allah, M.; Olsen, M.S.; Cairns, J.E. Translating high-throughput phenotyping into genetic gain. *Trends Plant Sci.* 2018, *23*(5),451-466: doi: 10.1016/j.tplants.2018.02.001.

Ashraf, M. Inducing drought tolerance in plants: Recent advances. *Biotechnol. Adv*. 2010, 28(1), 169–183. doi: 10.1016/j.biotechadv.2009.11.005.

Ayenan, M.A.T.; Danquah, A.; Hanson, P.; Ampomah-Dwamena, C.; Sodedji, F.A.K..; Asante, I.K.; Danquah, E.Y. Accelerating breeding for heat tolerance in tomato (Solanum lycopersicum L.): An integrated approach. *J. Agron*. 2019, *9*(11), 720: doi: 10.3390/agronomy9110720.

Badu-Apraku, B.; Hunter, R.B.; Tollenaar, M. Effect of temperature during grain filling on whole plant and grain yield in maize. (*Zea mays L.*). *Can. J. Plant Sci*. 1983, 63(2), 357–363. doi: 10.4141/cjps83-040.

Badu-Apraku, B.; Fakorede, M.A.B. Advances in genetic enhancement of early and extra-early maize for sub-Saharan Africa. *Adv. Genet. Enhanc*. 2017. 1–604. doi: 10.1007/978-3-319-64852-1.

Badu-apraku, B.; Fakorede, M.A.B.; Nelimor, C.; Osuman, A.S. Recent advances in breeding maize for drought , heat and combined heat and drought stress tolerance in sub-Saharan Africa. *Cabi Rev.* 2023: doi: https://doi.org/10.1079/cabireviews.2023.0011.

Bänziger, M.; Cooper, M. Breeding for low input conditions and consequences for participatory plant breeding examples from tropical maize and wheat. *Euphytica*. 2001, *122*, 503-519: doi: 10.1023/A:1017510928038.

Barnabás, B.; Jäger, K.; Fehér, A. The effect of drought and heat stress on reproductive processes in cereals. *Plant Cell Environ*, 2008.*31*(1), 11-38: doi: 10.1111/j.1365-3040.2007.01727.x.

Basavaraj, P.S.; Rane, J. Avenues to realize potential of phenomics to accelerate crop breeding for heat tolerance. *Plant Physiolo. Reports*. 2020, *25*, 594-610. doi: 10.1007/s40502-020-00552-2.

Bassu, S.; Brisson, N.; Durand, J.L.; Boote, K.; Lizaso, J.; Jones, J.W.; Rosenzweig, C.; Ruane, A.C.; Adam, M.; Baron, C.; Basso, B. How do various maize crop models vary in their responses to climate change factors? *Glob Chang Biol*, 2014. *20*(7). 2301-2320: doi: 10.1111/gcb.12520.

Beck, D.L.; Vasal, S.K.; Crossa, J. Heterosis and combining ability among subtropical and temperate intermediate-maturity maize germplasm. *Crop Sci.* 1991, *31*(1), 68-73. doi: 10.2135/cropsci1991.0011183x002600010017x.

Ben-Asher, J.; Garcia y Garcia, A.; Hoogenboom, G. Effect of high temperature on photosynthesis and transpiration of sweet corn (*Zea mays L. var. rugosa*). *Photosynthetica*. 2008, *46*, 595-603: doi: 10.1007/s11099-008-0100-2.

Betran, F.J.; Mayfield, K.; Isakeit, T.; Menz, M.; Breeding maize exotic germplasm. In Plant breeding: *Arnel R. Hallauer Int. Sym.* 2006, 352-367. doi: 10.1002/9780470752708.ch26.

Beyene, Y. Implimentation of new tools and technologies in the AGG-maize projest. Product Profile based breeding for increased genetic gain. 2022.

Beyene, Y.; Semagn, K.; Crossa, J.; Mugo, S.; Atlin, G.N.; Tarekegne, A.; Meisel, B.; Sehabiague, P.; Vivek,B.S.; Oikeh, S.; Alvarado, G.; Machida, L.; Olsen, M.; Prasanna, B.M.; Bänziger, M. Improving maize grain yield under drought stress and non-stress environments in sub-saharan africa using marker-assisted recurrent selection. *Crop Sci*. 2016, 56(1), 344–353. doi: 10.2135/cropsci2015.02.0135.

Beyene, Y.; Gowda, M.; Suresh, L.M.; Mugo, S.; Olsen, M.; Oikeh, S.O.; Juma, C.; Tarekegne, A.; Prasanna, B.M. Genetic analysis of tropical maize inbred lines for resistance to maize lethal necrosis disease. *Euphytica*, 2017.*213*, 1-13: doi: 10.1007/s10681-017-

Cairns, J.E.; Crossa, J.; Zaidi, P.H.; Grudloyma, P.; Sanchez, C.; Araus, J.L.; Thaitad, S.; Makumbi, D.; Magorokosho, C.; Bänziger, M.; Menkir, A. Identification of drought, heat, and combined drought and heat tolerant donors in maize. *Crop Sci*, 2013. *53*(4),1335-1346. doi: 10.2135/cropsci2012.09.0545.

Cairns, J.E.; Prasanna, B.M. Developing and deploying climate-resilient maize varieties in the developing world. *Curr. Opin. Plant Biol.* 2018. *45*, 226-230: doi: 10.1016/j.pbi.2018.05.004.

Casaretto, J.A.; El-Kereamy, A.; Zeng, B.; Stiegelmeyer, S.M.; Chen, X.; Bi, Y.M.; Rothstein, S.J. Expression of OsMYB55 in maize activates stress-responsive genes and enhances heat and drought tolerance. *BMC genomics*. 2016., *17*(1),1-15: doi: 10.1186/s12864-016-2659-5.

Chaikam, V.; Molenaar, W.; Melchinger, A.E.; Prasanna, B.M. Doubled haploid technology for line development in maize: Technical advances and prospects. *Theor. Appl. Genet.* 2019, 132(12): 3227–3243. doi: 10.1007/s00122-019-03433-x.

Chaikam, V.; Prasanna, B.M. Doubled Haploid Technology for Rapid and Efficient Maize Breeding. In *Accelerated Plant Breeding, Volume 1, Cereal Crops. 2020,* 257-292. Cham: Springer International Publishing.

Chapman, S.C.; Chakraborty, S.; Dreccer, M.F.; Howden, S.M. Plant adaptation to climate change—opportunities and priorities in breeding. *Crop Pasture Sci*. 2012, *63*(3), 251-268: doi: 10.1071/CP11303.

Chaudhary, S.; Devi, P.; Bhardwaj, A.; Jha, U.C.; Sharma, K.D.; Prasad, P.V.; Siddique, K.H.;

Bindumadhava, H.; Kumar, S; Nayyar, H. Identification and characterization of contrasting genotypes/cultivars for developing heat tolerance in agricultural crops: Current status and prospects. *Front Plant Sci*, 2020, *11*, 587264: doi: 10.3389/fpls.2020.587264.

Chen, J.; Xu, W.; Velten, J.; Xin, Z.; Stout, J. Characterization of maize inbred lines for drought and heat tolerance. *J. Soil Water Conserv.* 2012, 67(5), 354-364: doi: 10.2489/jswc.67.5.354.

Chikwati, E. 2022. Seed-Co lanches new maize varieties. https://www.herald.co.zw/seed-co-launches-new-maize-varieties/

Collins, N.C.; Tardieu, F.; Tuberosa, R. Quantitative trait loci and crop performance under abiotic stress: where do we stand?. *J. Plant Physiol.* 2008. *147*(2), pp.469-486. doi: 10.1104/pp.108.118117.

Corazza, T.C.; Carvalho, I.R.; Hutra, D.J.; Bradebon, L.C.; da Rosa Sarturi, M.V.; da Rosa, J.A.G.; Szareski, V.J. Maize breeding for abiotic stress tolerance: An alternative to face climate changes. *Agron. Sci. Biotechnol.* 2020. *6*, 1-13: doi: 10.33158/ASB.r119.v6.2020.

Crossa, J.; Pérez-Rodríguez, P.; Cuevas, J.; Montesinos-López, O.; Jarquín, D.; De Los Campos, G.; Burgueño, J.; González-Camacho, J.M.; Pérez-Elizalde, S.; Beyene, Y.; Dreisigacker, S. Genomic selection in plant breeding: methods, models, and perspectives. *Trends Plant Sci*. 2017, *22*(11), 961-975 : doi: 10.1016/j.tplants.2017.08.011.

Da Rosa, T.C.; Carvalho, I.R.; Hutra, D.J.; Bradebon, L.C.; da Rosa Sarturi, M.V.; da Rosa, J.A.G.; Szareski, V.J. Maize breeding for abiotic stress tolerance: An alternative to face climate changes. *J Crop Sci Biotechnol.2020, 6*, 1-13 : https://doi.org/10.33158/ASB.r119.v6.2020

Dinler, B.; Demir, E.; Kompe, Y. Regulation of auxin, abscisic acid and salicylic acid levels by ascorbate application under heat stress in sensitive and tolerant maize leaves. *Acta Biologica Hungarica*, 2014. *65*(4),469-480: doi: 10.1556/ABiol.65.2014.4.10.

Dinesh, A.; Patil, A.; Zaidi, P.H.; Kuchanur, P.H.; Vinayan, M.T.; Seetharam, K. Line x testers analysis of tropical maize inbred lines under heat stress for grain yield and secondary traits. *Maydica*. 2016. 61(1): 3–6.

Driedonks, N.; Rieu, I.; Vriezen, W.H.. Breeding for plant heat tolerance at vegetative and reproductive stages. *Plant Reprod.* 2016, *29(1-2)*, 67-79: doi: 10.1007/s00497-016-0275-9.

Duvick, D.N. The contribution of breeding to yield advances in maize *(Zea mays L.*). *Adv. Agron.* 2005, *86*, 83-145: doi: 10.1016/S0065-2113(05)86002-X.

Duvick, D.N.; Cassman, K.G. Post–green revolution trends in yield potential of temperate maize in the North-Central United States. *Crop Sci.* 1999, *39*(6), 1622-1630: doi: 10.2135/cropsci1999.3961622x.

Dwivedi, S.L.; Ceccarelli, S.,; Blair, M.W.; Upadhyaya, H.D.; Are, A.K.; Ortiz, R. Landrace germplasm for improving yield and abiotic stress adaptation. *Trends Plant Sci.* 2016, 21(1), 31-42: doi: 10.1016/j.tplants.2015.10.012.

Dwivedi, S.L.; Crouch, J.H.; Mackill, D.J.; Xu, Y.; Blair, M.W.; Ragot, M.; Upadhyaya, H.D.; Ortiz, R. The molecularization of public sector crop breeding: progress, problems, and prospects. *Adv. Agron.* 2007. *95*, 163-318: doi: 10.1016/S0065-2113(07)95003-8.

Eathington, S.R.; Crosbie, T.M.; Edwards, M.D.; Reiter, R.S.; Bull, J.K. Molecular markers in a commercial breeding program. *Crop Sci.* 2007, 47, 154: doi: 10.2135/cropsci2007.04.0015IPBS.

Edmeades, G. O.; Bänziger, M.; Elings, A.; Chapman, S.C.; Ribaut, J.M. "Recent advances in breeding for drought tolerance in maize." In Applications of Systems Approaches at the Field Level: Volume 2 Proceedings of the Second International Symposium on Systems Approaches for Agricultural Development, held at IRRI, Los Baños, Philippines, 6–8 December 1995, 63-78. Springer Netherlands, 1997.

Edmeades, G.O.; Trevisan, W.; Prasanna, B.M.; Campos, H. Tropical Maize (*Zea mays* L.). In: Genetic Improvement of Tropical Crops. Springer, Cham. 2017, 1, 57-109, https://doi.org/10.1007/978-3-319-59819-2_3

Edreira, J.I.R.; Mayer, L.I.; Otegui, M.E.; Heat stress in temperate and tropical maize hybrids: Kernel growth, water relations and assimilate availability for grain filling. *F. Crop. Res.* 2014, *166*, 162-172. https://doi.org/10.1016/j.fcr.2014.06.018

Edreira, J.I.R.; Mayer, L.I.; Otegui, M.E.; Heat stress in temperate and tropical maize hybrids: Differences in crop growth, biomass partitioning and reserves use. *F. Crop. Res.* 2012, 130, *87-98* : doi.org/10.1016/j.fcr.2014.06.018

El-Sappah, A. H.; Rather, S. A.; Wani, S. H.; Elrys, A. S.; Bilal, M.; Huang, Q.; Zahoor Ahmad

DaMohamed M. A.; Soaud, E.N., Koul., E.M; Mir, R.R.; Yan, K.Y.; Abbas, M.J. Heat stress-mediated constraints in maize (Zea mays) production: challenges and solutions. *Front. Plant Sci*. 2022 , 13, 879366. https://doi.org/10.3389/fpls.2022.879366.

Fahad, S.; Bajwa, A.A..; Nazir, U.; Anjum, S.A.; Farooq, A.; Zohaib, A.; Sadia, S.; Nasim, W.; Adkins, S.; Saud, S.; Ihsan, M.Z. Crop production under drought and heat stress: plant responses and management options. *Front. Plant Sci,* 2017.18, 1147: doi: 10.3389/fpls.2017.01147.

FAOSTAT. 2021. World Food and Agriculture – Statistical Yearbook 2021.

Fasahat, P.; Rajabi, A.; Rad, J.M.; Derera, J. Principles and utilization of combining ability in plant breeding. *Biometrics Biostat. Int. J.* 2016. *4*(1), 1-22: doi: 10.15406/bbij.2016.04.00085.

Frey, F.P.; Presterl, T.; Lecoq, P.; Orlik, A.; Stich, B. First steps to understand heat tolerance of temperate maize at adult stage: identification of QTL across multiple environments with connected segregating populations. *Theor. Appl. Genet*. 2016, *129(5),* 945-961. doi: 10.1007/s00122-016-2674-6.

Frova, C.; Sari-Gorla, M. Quantitative trait loci (QTLs) for pollen thermotolerance detected in maize. *MGG Mol. Gen. Genet.* 1994, *245(4),* 424-430: https://doi.org/10.1007/BF00302254

Frova, C.; Caffulli, A.; Pallavera, E. Mapping quantitative trait loci for tolerance to abiotic stresses in maize. *J. Exp. Zool.* 1998, *282*(1-2), 164-170: doi: 10.1002/(sici)1097-010x(199809/10)282:1/2<164::aid-jez18>3.0.co;2-u.

Gao, J.; Wang, S.; Zhou, Z.; Wang, S.; Dong, C.; Mu, C.; Song, Y.; Ma, P.; Li, C.; Wang, Z.; and He, K.. Linkage mapping and genome-wide association reveal candidate genes conferring thermotolerance of seed-set in maize. *J Exp Bot*, 2019. *70* (18), 4849-4864: https://doi.org/10.1093/jxb/erz171

Galluzzi, G.; Seyoum, A.; Halewood, M.; Lopez Noriega, I.; Welch, E.W. The role of genetic resources in breeding for climate change: The case of public breeding programmes in eighteen developing countries. *Plants*. 2020, *9*(9), 1129: doi: 10.3390/plants9091129.

Gasura, E.; Setimela, P.; Souta, C. Evaluation of the performance of sorghum genotypes using GGE biplot. Can. J. Plant Sci. 2015, 95(6), 1205–1214. https://doi.org/10.4141/cjps-2015-119

Gazala, P.; P. Kachanur, P.; Zaidi, B.; Amnkumar, P.; Ayyanagouda, et al. 2018. Combining ability and heterosis for heat stress tolerance in maize (Zea mays L.). Bangladesh J. Plant Breed. Genet. 21(1): 21–28. doi: 10.3329/bjpbg.v21i1.17045.

Gedil, M.; Menkir, A. An integrated molecular and conventional breeding scheme for enhancing genetic gain in maize in Africa. *Front. Plant Sci.* 2019, 10, 1430: doi: 10.3389/fpls.2019.01430.

Georges, F.; Ray, H. Genome editing of crops: A r enewed opportunity for food security. *GM Crops and Food*, 2017. *8*(1), 1-12: https://doi.org/10.1080/21645698.2016.1270489

Gill, S.S.; Tuteja, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* 2010. *48*(12), 909-930: doi: 10.1016/j.plaphy.2010.08.016.

Govindaraj, M.; Pattanashetti, S.K.; Patne, N.; Kanatti, A.A.; Ciftci, Y.O. Breeding cultivars for heat stress tolerance in staple food crops. *Next Gener. Plant Breed.* 2018, 45-74: doi: 10.5772/intechopen.76480.

Grover, A.; Mittal, D.; Negi, M.; Lavania, D. Generating high temperature tolerant transgenic plants: Achievements and challenges. *Plant Sci.* 2013, *205*,.38-47: doi: 10.1016/j.plantsci.2013.01.005.

Gupta, P.K.; Balyan, H.S.; Gahlaut, V. QTL analysis for drought tolerance in wheat: Present status and future possibilities. *J. Agron.* 2017, *7*(1), 5: doi: 10.3390/agronomy7010005.

Han, G. 2021. Gene Editing Technology Used to Modify Traits

Hasanuzzaman, M.; Nahar, K.; Alam, M.M.; Roychowdhury, R.; Fujita, M. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *Int. J. Mol. Sci*. 2013, *14*(5), 9643-9684: https://doi.org/10.3390/ijms14059643

Hemantaranjan, A. Heat Stress Responses and Thermotolerance. *Adv. Plants Agric. Res.* 2014, 1(3): 62–70. doi: 10.15406/apar.2014.01.00012.

Herrero, M.P.; Johnson, R.R. High temperature stress and pollen viability of maize. *Crop Sci.* 1980, *20*(6), 796-800: doi: 10.2135/cropsci1980.0011183x002000060030x.

Holland, J.B. Breeding: Incorporation of exotic germplasm. *Encyclopedia of Plant and Crop Science. Marcel Dekker,* 2004, 222-224: doi=e597fc3d3b6389b03e1b257d4bf9674dd6c540b9

Hussain, H.A.; Men, S.; Hussain, S.; Chen, Y.; Ali, S.; Zhang, S.; Zhang, K.; Li, Y.; Xu, Q.; Liao, C.;

Wang, L. Interactive effects of drought and heat stresses on morpho-physiological attributes, yield, nutrient uptake and oxidative status in maize hybrids. *Sci. Re.* 2019, *9*(1),.3890: doi: 10.1038/s41598-019-40362-7.

Iglesias, C.A. Efficiency of S2 recurrent selection for improvement of exotic and semi-exotic populations in maize (Zea mays L.). 1989. Iowa State University. PhD

Inghelandt, D.V.; Frey, F.P.; Ries, D.; Stich, B. QTL mapping and genome-wide prediction of heat tolerance in multiple connected populations of temperate maize. *Sci. Re.* 2019, *9*(1), 1-16: doi: 10.1038/s41598-019-50853-2.

Jagtap, A.B.; Vikal, Y.; Johal, G.S. Genome-wide development and validation of cost-effective KASP marker assays for genetic dissection of heat stress tolerance in maize. *Int. J. Mol. Sci.* 2020, 21(19), 7386: ***https://doi.org/10.3390/ijms21197386***

Janni, M.; Gullì, M.; Maestri, E.; Marmiroli, M.; Valliyodan, B.; Nguyen, H.T.; Marmiroli, N. Molecular and genetic bases of heat stress responses in crop plants and breeding for increased resilience and productivity. *J. Exp. Bot.* 2020, 71(13): 3780–3802. doi: 10.1093/jxb/eraa034.

Jodage, K.; Kuchanur, P.H.; Zaidi, P.H.; Patil, A.; Seetharam, K.; Vinayan, M.T.; Arunkumar, B. Genetic analysis of heat adaptive traits in tropical maize (Zea mays L.). *Int. J. Curr. Microbiol. Appl. Sci.* 2017, 7(1), 3237–3246: doi: 10.20546/ijcmas.2018.701.387.

Jodage, K.; Kuchanur, P.H.; Zaidi, P.H.; Patil, A.; Seetharam, K.; Vinayan, M.T.; Arunkumar, B. Genetic Analysis of Heat Adaptive Traits in Tropical Maize (*Zea mays* L.). *Int. J. Curr. Microbiol. Appl. Sci.* 2018, *7*, 3237–3246. https://doi.org/10.20546/ijcmas.2018.701.387.

Kandel, M.; Ghimire, S.K.; Ojha, B.R.; Shrestha, J. Analysis of genetic diversity among the maize inbred lines (Zea mays L.) under heat stress condition. *J. Maiz. Res. Dev*, 2017. *3*(1), 86-97: doi: 10.3126/jmrd.v3i1.18925.

Kandel, M.; Ghimire, S.K.; Ojha, B.R.; Shrestha, J. Correlation and path coefficient analysis for grain yield and its attributing traits of maize inbred lines (Zea mays L.) under heat stress condition. *Int. J. Agric. Environ. Food Sc.* 2018, *2*(4), 124-130: doi: 10.31015/jaefs.18021.

Kassie, G.T.; Erenstein, O.; Mwangi, W.M.; La Rovere, R.; Setimela, P.S.; Langyintuo, A.S. Characterization of maize production in Southern Africa: synthesis of CIMMYT/DTMA household level farming system surveys in Angola, Malawi, Mozambique, Zambia and Zimbabwe. 2012.

Kaushal, N.; Bhandari, K.; Siddique, K.H.; Nayyar, H. Food crops face rising temperatures: An overview of responses, adaptive mechanisms, and approaches to improve heat tolerance. *Cogent Food Agric.* 2016, *2*(1), 1134380: doi: 10.1080/23311932.2015.1134380.

Khan, S.U., J.U. Din, A. Qayyum, N.E. Jan, and M.A. Jenks. Heat tolerance indicators in Pakistani wheat (Triticum aestivum L.) genotypes. *Acta Bot. Croat.* 2015, 74(1): 109–121. doi: 10.1515/botcro-2015-0002.Khan, S.; Anwar, S.; Ashraf, M.Y.; Khaliq, B.; Sun, M..; Hussain, S.; Gao, Z.Q.; Noor, H.; and Alam, S. Mechanisms and adaptation strategies to improve heat tolerance in rice. A review. *Plants*, 2019, *8*(11), 1-21: doi:10.3390/plants8110508

Khodarahmpour, Z.; Choukan, R.; Bihamta, M.R.; Majidi, H.E. Determination of the best heat stress tolerance indices in maize (*Zea mays L.*) inbred lines and hybrids under Khuzestan province conditions. *J. Agric. Sci. Technol*. 2011. 13(1), 111–121.

Koirala, K.B.; Tripathi, M.P.; Seetharam, K.; Vinayan, M.T.; and Zaidi, P.H. Field evaluation of heat stress-resilient maize hybrids for improved and stable maize production in Nepal. *SAARC J. Agric*. 2021, 19(1), 27–43. doi: 10.3329/sja.v19i1.54776.

Kumar, S.; Gupta, D; Nayyar, H. Comparative response of maize and rice genotypes to heat stress: status of oxidative stress and antioxidants. *Acta Physiol. Plant.* 2012, *3*4(1),75–86. doi: 10.1007/s11738-011-0806-9.

Lafarge, T.; Bueno, C.; Frouin, J. ; Jacquin, L.; Courtois, B.; Ahmadi, N. Genome-wide association analysis for heat tolerance at flowering detected a large set of genes involved in adaptation to thermal and other stresses. *PLoS One*, 2017, *12*(2), 1–27: doi: 10.1371/journal.pone.0171254.

Lamaoui, M.; Jemo, M.; Datla, R.; Bekkaoui, F. Heat and drought stresses in crops and approaches for their mitigation. *Front. Chem.* 2018, 6, 26: https://doi.org/10.3389/fchem.2018.00026

Li, Z.F.; Zhang, Y.C; Chen, Y.Q. miRNAs and lncRNAs in reproductive development. *Plant Sci.* 2015, *238*,46-52: doi: 10.1016/j.plantsci.2015.05.017.

Lin, H.; Chen, Y.; Zhang, H.; Fu, P.; Fan, Z. Stronger cooling effects of transpiration and leaf physical traits of plants from a hot dry habitat than from a hot wet habitat. *Funct. Ecol.* 2017.*31*(12), 2202-2211: doi: 10.1111/1365-2435.12923.

Lipiec, J.; Doussan, C.; Nosalewicz, A.; Kondracka. K. Effect of drought and heat stresses on plant growth and yield: A review. Int. Agrophysics. 2013, 27(4), 463–477: doi: 10.2478/intag-2013-0017.

Liu, S.; Qin, F. Genetic dissection of maize drought tolerance for trait improvement. *Mol Breeding.* 2021. 41, 8 : https://doi.org/10.1007/s11032-020-01194-w

Lobell, D.B.; Bänziger, M.; Magorokosho, C; Vivek, B. Nonlinear heat effects on African maize as evidenced by historical yield trials. Nat. Clim.Chang, 2011, 1(1), 42–45, doi: 10.1038/nclimate1043.

Lobell, D.B.; Sibley, A.; Ivan Ortiz-Monasterio, J. Extreme heat effects on wheat senescence in India. *Nat. Clim. Chang.* 2012.*2*(3), 186-189: doi: 10.1038/nclimate1356.

Longmei, N.; Gill, G.K.; Kumar, R.; Zaidi, P.H.; Pal, L. Identification of heat tolerant hybrids based on morphological traits and selection indices. *Int. J. Curr. Microbiol. Appl. Sci*.2020. 9(6), 1814–1823. doi: 10.20546/ijcmas.2020.906.226.

Longmei, N.; Gill, G.K.; Zaidi, P.H.; Kumar, R.; Nair, S.K.; Hindu, V.; Vinayan, M.T.; Vikal, Y. Genome wide association mapping for heat tolerance in sub-tropical maize. *BMC genomics*. 2021, *22*(1), 1-14. doi: 10.1186/s12864-021-07463-y.

Ludlow, M.M.; Muchow, R.C. A critical evaluation of traits for improving crop yields in water-limited environments. *Adv. Agric.* 1990. *43*, 107-153: doi: 10.1016/S0065-2113(08)60477-0.

Lutt, N.; Jeschke, M.; Strachan, S.D. High night temperature effects on corn yield. *DuPont Pioneer Agro Scie. Crop Insights*, 2016, *26*, 16.

Maazou, A.R.S.; Tu, J.; Qiu, J.; Liu, Z. Breeding for drought tolerance in maize (Zea mays L.). *Am. J. Plant Sci.* 2016, *7*(14), 1858: doi: 10.4236/ajps.2016.714172.

Maestri, E.; Klueva, N.; Perrotta, C.; Gulli, M.; Nguyen, H.T.; Marmiroli, N. Molecular genetics of heat tolerance and heat shock proteins in cereals. *Plant Mol. Biol.* 2002, *48*, 667-681: https://doi.org/10.1023/A:1014826730024

Mahmood, S.; Wahid, A.; Javed, F.; Basra, S.M. Heat stress effects on forage quality characteristics of maize (Zea mays) cultivars. *nt. J. Agric. Biol.* 2010, 12(5): 701–706. 10–158/HUT/2010/12–5–

701–706

Makumbi, D.; Diallo, A.; Kanampiu, F.; Mugo, S.; Karaya, H. Agronomic performance and gen- otype x environment interaction of herbicide-resistant maize varieties in Eastern Africa. *Crop Sci*. 2015, 55(2), 540–555. https://doi.org/10.2135/ cropsci2014.08.0593

Mallikarjuna, M.G.; Bhat, J.S.; Hossain, F.; Veeraya, P.; Tyagi, A.; Karjagi, C.G.; Lohithaswa, H.C. Genetic enhancement of heat tolerance in maize through conventional and modern strategies. Heat stress in food grain crops: Plant breeding and omics research, 2020, 28-66.

Manoj, K.; Ghimire, S.K.; Jiban, S. Mechanisms of heat stress tolerance in maize. *Azarian J. Agric.*2018. *5*(1), 20-27:

Manoj, K.; Ghimire, S.K.; Ojha, B.R.; Shrestha, J. Genetic diversity for heat tolerant related traits in maize inbred lines. *Agricultura*, 2018.1 (2), *105*-106: doi: 10.15835/agrisp.v105i1-2.13038.

Manoj, K.; Ghimire, S.K.; Ojha, B.R.; Shrestha, J. Evaluation of heat stress tolerance indices in maize inbred lines. *Malays. J. Fundam. Appl. Sci.* 2019, *4*(2), 57-68: https://journal.unisza.edu.my/myjas

Manavalan, L.P.; Guttikonda, S.K.; Phan Tran, L.S.; Nguyen, H.T. Physiological and molecular approaches to improve drought resistance in soybean. *Plant Cell Physiol*, 2009. *50*(7), 1260-1276. doi: 10.1093/pcp/pcp082.

Marzec, M.; Brąszewska-Zalewska, A.; Hensel, G. Prime editing: A new way for genome editing. *Trends Cell Biol.* 2020. *30*(4),257-259: doi: 10.1016/j.tcb.2020.01.004.

Matova, P.M.; Kamutando, C.N.; Mutari, B.; Magorokosho, C.; Labuschagne, M. Adaptability and Stability Analysis of Commercial Cultivars, Experimental Hybrids and Lines under Natural Fall Armyworm Infestation in Zimbabwe Using Different Stability Models. *J. Agron, 2022. 12(7),* 1724: doi: 10.3390/agronomy12071724.

McNellie, J.P.; Chen, J.; Li, X.; Yu, J. Genetic mapping of foliar and tassel heat stress tolerance in maize. *Crop Science*. 2018, *58*(6), 2484-2493: https://doi.org/10.2135/cropsci2018.05.0291

McMillen, M.S.; Mahama, A.A.; Sibiya, J.; Lübberstedt, T.; Suza, W.P.; Improving drought tolerance in maize: Tools and techniques. *Frontiers in Genetics,* 2022, *13,*1-13: doi: 10.3389/fgene.2022.1001001.

Meena, R.S.; Lal, R.; Yadav, G.S. Long-term impacts of topsoil depth and amendments on soil physical and hydrological properties of an Alfisol in central Ohio, USA. *Geoderma*, 2020. *363*: doi: 10.1016/j.geoderma.2019.114164.

Meseka, S.; Menkir, A.; Bossey, B.; Mengesha, W. Performance assessment of drought tolerant maize hybrids under combined drought and heat stress. *J. Agron.* 2018.*8*(12), 274: doi: 10.3390/agronomy8120274.

Mhike,X. Use of morpho-physiological traits to enhance breeding for drought tolerance in tropical aize, 2013, Makerere University, Doctorate thesis.

Michelini, L.A.; Hallauer, A.R. Evaluation of exotic and adapted maize (*Zea mays L.*) germplasm crosses. *Maydica.* 1993, 38(4), 275–282. doi: 10.31274/rtd-180813-12230.

Mobin, M.; Khan, M.N.; Abbas, Z.K.; Ansari, H.R.; Al-Mutairi, K.A. Significance of sulfur in heat stressed cluster bean (Cymopsis tetragonoloba L. Taub) genotypes: responses of growth, sugar and antioxidative metabolism. *Arch. Agron. Soil Sci.* 2017, *63*(2), 288-295: doi: 10.1080/03650340.2016.1196811.

Mukaro, R.; Kamutando, C.N.; Magorokosho, C.; Mutari, B.; Zaidi, P.H.; Kutywayo, D.; Sibiya, J. Genetic potential of tropically adapted exotic maize (Zea mays L.) heat-tolerant donor lines in sub-tropical breeding programs. *J. Agron*, 2023. *13*(8), 1-6: https://doi.org/10.3390/agronomy13082050

Mukaro et. al unpublished: Grain yield stability of early maturity commercial maize hybrids under heat stress prone agro-ecologies of Zimbabwe.

Muraya, M.M.; Ndirangu, C.M.; Omolo, E.O. Heterosis and combining ability in diallel crosses involving maize (Zea mays) S1 lines. *A Aust. J. Exp. Agric*, 2006. *46*(3), 387-394: doi: 10.1071/EA03278.

Nadeem, M.; Li, J.; Wang, M.; Shah, L.; Lu, S.; Wang, X.; Ma, C.; Unraveling field crops sensitivity to heat stress: Mechanisms, approaches, and future prospects. *J. Agron.* 2018. *8*(7), 128: doi: 10.3390/agronomy8070128.

Naveed, M.; Ahsan, M.; Akram, H.M.; Aslam, M.; and Ahmed, N. Genetic effects conferring heat tolerance in a cross of tolerant× susceptible maize (Zea mays L.) genotypes. *Front. Plant Sci.* 2016, *7*, 1-12: doi: 10.3389/fpls.2016.00729.

Ndhlela, T.; Herselman, L.; Magorokosho, C.; Setimela, P.; Mutimaamba, C.; Labuschagne, M. Genotype× environment interaction of maize grain yield using AMMI biplots. *Crop Sci.* 2014. *54*(5), 1992-1999: doi: 10.2135/cropsci2013.07.0448.

Ndoro, O. F. Use of exotic germplasm to enhance the performance of local maize, *2019.* University of the Free State, Doctoral dissertation.

Nelimor, C.; Badu-Apraku, B.; Tetteh, A.Y.; N'guetta, A.S. Assessment of genetic diversity for drought, heat and combined drought and heat stress tolerance in early maturing maize landraces. *Plants*. 2019.*8*(11), 1-9: doi: 10.3390/plants8110518.

Nelimor, C.; Badu-Apraku, B.; Tetteh, A.Y.; Garcia-Oliveira, A.L.; N'guetta, A.S.P. Assessing the potential of extra-early maturing landraces for improving tolerance to drought, heat, and both combined stresses in maize. *J. Agron.* 2020. *10(3): 1–26. doi: 10.3390/agronomy10030318.*

Newell, M.A.; Jannink, J. Genomic Selection in Plant Breeding.in Breeding Methods in Molecular Biology. 2014, 1145: 117–130. doi: 10.1007/978-1-4939-0446-4.

Ni, Z.; Li, H.; Zhao, Y.; Peng, H.; Hu, Z.; Xin, M.; Sun, Q. Genetic improvement of heat tolerance in wheat: recent progress in understanding the underlying molecular mechanisms. *Crop J.*, 2018. *6*(1), 32-41: doi: 10.1016/j.cj.2017.09.005.

Noor, J.J.; Vinayan, M.T.; Umar, S.; Devi, P.; Iqbal, M.; Seetharam, K.; Zaidi, P.H. Morpho-physiological traits associated with heat stress tolerance in tropical maize ('Zea mays' L.) at reproductive stage. *Aust. J. Crop Sci.* 2019, *13*(4), 536-545: doi: 10.21475/ajcs.19.13.04.p1448.

Nzuve, F.; Githiri, S.; Mukunya, D.M.; Gethi, J. Analysis of genotype x environment interaction for grain yield in maize hybrids. J. Agric. Sci. 2013, 5(11): doi: 10.5539/jas.v5n11p75.

Patil, S.V.; Doggalli, G.; Kuchanur, P.H.; Zaidi, P.H.; Patil, A.; Arunkumar, B.; Vinayana, M.T.; Tembhurne, B.V.; Suma, T.C.; Seetharam, K. Assessing combining ability of doubled haploid maize (Zea mays L.) breeding lines for grain yield and yield components under heat stress condition. *Int. j. plant soil sci*. 2022. 34(22), 510–518. doi: 10.9734/IJPSS/2022/v34i2231403.

Peverelli, M.C.; Rogers, W.J. Heat stress effects on crop performance and tools for tolerance breeding. *Ciencias Agrar*. 2013. *45*(2), pp.349-368: http://www.redalyc.org/articulo.oa?id=382837655001.

Prasanna, B.M. Diversity in global maize germplasm: Characterization and utilization. J. Biosci. 2012. 37(5): 843–855. doi: 10.1007/s12038-012-9227-1.

Prasanna, B.M.; Cairns, J.E.; Zaidi, P.H.; Beyene, Y.; Makumbi, D.; Gowda, M.; Magorokosho, C.; Zaman-Allah, M.; Olsen, M.; Das, A.; Worku, M.; Beat the stress: breeding for climate resilience in maize for the tropical rainfed environments. *Theor. Appl. Genet.* 2021, *134*(6).1729-1752: doi: 10.1007/s00122-021-03773-7.

Prasanna, B.M.; Burgueño, J.; Beyene, Y.; Makumbi, D.; Asea, G.; Woyengo, V.; Tarekegne, A.; Magorokosho, C.; Wegary, D.; Ndhlela, T.; Zaman-Allah, M et al. Genetic trends in CIMMYT's tropical maize breeding pipelines. *Scie Rep*, 2022, *12*(1), p.20110. https://doi.org/10.1038/s41598-022-24536-4

Queitsch, C.; Hong, S.W.; Vierling, E.; and Lindquist, S. Heat shock protein 101 plays a crucial role in thermotolerance in Arabidopsis. *The Plant Cell*. 2000, *12*(4), 479-492: doi: 10.1105/tpc.12.4.479.

Rai, A.; Mahata, D.; Lepcha, E.; Nandi, K.; Mukherjee, P.K.; A review on management of weeds in maize (Zea mays L.). *Int. J. Curr. Microbiol. App. Sci*, 2018. *7*(8), 2906-2922: doi: 10.20546/ijcmas.2018.708.308.

Ribaut, J.M.; De Vicente, M.C.; Delannay, X.; Molecular breeding in developing countries: challenges and perspectives. *Curr. Opin. Plant Biol*. 2010.*13*(2), 213-218: doi: 10.1016/j.pbi.2009.12.011.

Richards, R.A.; Rawson, H.M.; and Johnson, D.A. Glaucousness in wheat: its development and effect on water-use efficiency, gas exchange and photosynthetic tissue temperatures. *Funct. Plant Biol.* 1986. *13*(4), 465-473. doi: 10.1071/pp9860465.

Rodríguez, M.; Canales, E.; Borrás-Hidalgo, O. Molecular aspects of abiotic stress in plants. *Biotecnol. Apl.* 2005. *22*(1), 1-10

Rowhani, P.; Lobell, D.B.; Linderman, M.; and Ramankutty, N. Climate variability and crop production in Tanzania. *Agric. For. Meteorol.* 2011. *151*(4), 449-460: doi: 10.1016/j.agrformet.2010.12.002.

Ruelland, E.; Zachowski,A. How plants sense temperature. *Environ. Exp. Bot. 2010.* 69(3) 225–232.

doi: 10.1016/j.envexpbot.2010.05.011.

Sabagh, A.E.; Hossain, A.; Iqbal, M.A.; Barutçular, C.; Islam, M.S.; Çiğ, F.; Erman, M.; Sytar, O.; Brestic, M.; Wasaya, A.; Jabeen, T. et . al. Maize adaptability to heat stress under changing climate. In *J. Plant Stress Physiol.* 2020. doi: 10.5772/intechopen.92396

Sandhu, K.S.; Singh, N.; Malhi, N.S. Some properties of corn grains and their flours I: Physicochemical, functional and chapati-making properties of flours. *Food Chem.* 2007.101(3), 938-946: doi: 10.1016/j.foodchem.2006.02.040.

Santantonio, N.; Atanda, S.A.; Beyene, Y.; Varshney, R.K.; Olsen, M.; Jones, E.; Roorkiwal, M.; Gowda, M.; Bharadwaj, C.; Gaur, P.M.; Zhang, X. Strategies for effective use of genomic information in crop breeding programs serving Africa and South Asia. *Front Plant Sci*. 2020. *11* (353).1-12: doi: 10.3389/fpls.2020.00353.

Setimela, P. S.; Crossa, J.; Bänziger, M. Targeting of early to intermediate maize hybrids for yield performance and stability using SREG model. *S Afr J Plant Soil*. 2010, 27(3), 207–216. https://doi.org/10.1080/02571862.2010.10639988

Seetharam, K.; Kuchanur, P.H.; Koirala, K.B.; Tripathi, M.P.; Patil, A.; Sudarsanam, V.; Das, R.R.; Chaurasia, R.; Pandey, K.; Vemuri, H.; Vinayan, M.T. Genomic regions associated with heat stress tolerance in tropical maize (Zea mays L.). *Sci. Rep.* 2021, *11*(1), 1-11: doi: 10.1038/s41598-021-93061-7.

Sehgal, A.; Sita, K.; Siddique, K.H.; Kumar, R.; Bhogireddy, S.; Varshney, R.K..; HanumanthaRao, B.; Nair, R.M.; Prasad, P.V.; Nayyar, H. Drought or/and heat-stress effects on seed filling in food crops: impacts on functional biochemistry, seed yields, and nutritional quality. *Front. Plant Sci.* 2018, *9*, 1-19. doi: 10.3389/fpls.2018.01705.

Shaibu, A.S.; Adnan, A.A.; Jibrin, J.M. Stability performance of extra early maturing maize (Zea mays L.) varieties under high and low nitrogen environments in Sudan Savanna. *Cogent Food Agric*. 2016, *2*(1), 1-12, doi: 10.1080/23311932.2016.1231456.

Shavrukov, Y.; Kurishbayev, A.; Jatayev, S.; Shvidchenko, V.; Zotova, L.; Koekemoer, F.; De Groot, S.; Soole, K.; Langridge, P. Early flowering as a drought escape mechanism in plants: how can it aid wheat production? *Front. Plant Sci*, 2017. *8*, 1-8 , doi: 10.3389/fpls.2017.01950.

Shi, T.Q.; Liu, G.N.; Ji, R.Y.; Shi, K.; Song, P.; Ren, L.J; Huang, H.; and Ji, X.J. CRISPR/Cas9-based genome editing of the filamentous fungi: The state of the art. *Appl. Microbiol. Biotechnol.* 2017. *101(20)*, 7435-7443: doi: 10.1007/s00253-017-8497-9.

Shikha, M.; Kanika, A.; Rao, A.R.; Mallikarjuna, M.G.; Gupta, H.S; Nepolean, T. Genomic selection for drought tolerance using genome-wide SNPs in maize. *Front. Plant Sci.* 2017. *8,*1-12: doi: 10.3389/fpls.2017.00550.

Shikha, K.; Shahi, J.P.; Vinayan, M.T.; Zaidi, P.H.; Singh, A.K.; Sinha, B. Genome-wide association mapping in maize: status and prospects. *Biotech*, 2021. *11*(5), 1-10, doi: 10.1007/s13205-021-02799-4.

Sibiya, J.; Tongoona, P.; Derera, J; van Rij, N. Genetic analysis and genotype× environment (G× E) for grey leaf spot disease resistance in elite African maize (Zea mays L.) germplasm. *Euphytica*, 2012. *185*, 349-362: doi: 10.1007/s10681-011-0466-2.

Sleper, J.A. and Bernardo, R. Recombination and genetic variance among maize doubled haploids induced from F 1 and F 2 plants. Theor. Appl. Genet. 2016. 1299 (12) 2429-2436: doi: 10.1007/s00122-016-2781-4.

Singh, B.; Salaria, N.; Thakur, K.; Kukreja, S.; Gautam, S.; Goutam, U. Functional genomic approaches to improve crop plant heat stress tolerance. *F1000Res, 2019, 1(8),* 1721: https://doi.org/10.12688%2Ff1000research.19840.1Sleper, J.A.;Bernardo, R.. Recombination and genetic variance among maize doubled haploids induced from F 1 and F 2 plants. *Theor. Appl. Genet.* 2016, *129*9 (12) 2429-2436: doi: 10.1007/s00122-016-2781-4.

Smith, K.L. *Ohio Agronomy Guide for Corn Production*; Ohio State University: Columbus, GA, USA, 1996.

Sserumaga, J.P.; Oikeh, S.O.; Mugo, S.; Asea, G.; Otim, M.; Beyene, Y.; Abalo, G.; Kikafunda, J. Genotype by environment interactions and agronomic performance of doubled haploids testcross maize (Zea mays L.) hybrids. *Euphytica,* 2016. *207*(2), 353-365: doi: 10.1007/s10681-015-1549-2.

Sultan, B.; and Gaetani, M. Agriculture in West Africa in the twenty-first century: Climate change and impacts scenarios, and potential for adaptation. *Front. Plant Sci.* 2016. *7,* 1–20. doi: 10.3389/fpls.2016.01262.

Tadesse, B.; Beyene, Y.; Das, B.; Mugo, S.N.; Olsen, M.; Oikeh, S.O.; Juma, C.; Labuschagne, M.; Prasanna, B.M. Combining ability and testcross performance of drought-tolerant maize inbred lines under stress and non-stress environments in Kenya. *Plant Breed*. 2017, 136(2): 197–205. doi: 10.1111/pbr.12464.

Tao, Z.Q.; Chen, Y.Q.; Chao, L.I.; Zou, J.X.; Peng, Y.A.N.; Yuan, S.F.; Xia, W.U.; Peng, S.U.I. The causes and impacts for heat stress in spring maize during grain filling in the North China Plain-A review. *J. Integr. Agric.* 2016. *15*(12), 2677-2687: doi: 10.1016/S2095-3119(16)61409-0.

Tao, F.; Zhang, Z. Adaptation of maize production to climate change in North China Plain: Quantify the relative contributions of adaptation options. *Eur. J. Agron*, 2010. *33*(2), 103-116: doi: 10.1016/j.eja.2010.04.002.

Tassawar, H.; Iftikhar, A.K; Zulfiqar, A. Study on gene action and combining abilities for thermotolerant ablilities of corn (Zea mays L.). *Int. J. Plant Prod.* 2012.1(1), 1-12: http://www.rsis.ir/journals/ijpp/datamag/47.pdf.

Tayade, R.; Nguyen, T.D.; Oh, S.A.; Hwang, Y.S.; Yoon, I.S.; Deshmuk, R.; Park,S.K. Effective strategies for enhancing tolerance to high-temperature stress in rice during the reproductive and ripening stages. *Plant Breed. Biotechnol*. 2018, 6(1), 1–18. https://doi.org/10.9787/PBB.2018.6.1.1

Telfer, P.; Edwards, J.; Bennett, D.; Ganesalingam, D.; Able, J.; Kuchel, H. A field and controlled environment evaluation of wheat (Triticum aestivum) adaptation to heat stress. *Field Crops Res.* 2018, *229*, 55-65: doi: 10.1016/j.fcr.2018.09.013.

Teixeira, E.I.; Fischer, G.; Van Velthuizen, H.; Walter, C.; Ewert, F. Global hot-spots of heat stress on agricultural crops due to climate change. *Agric. For. Meteorol.* 2013, *170*, 206-215: doi: 10.1016/j.agrformet.2011.09.002.

Teng, L.I.; Xue-peng, Z.H.A.N.G.; Qing, L.I.U.; Jin, L.I.U.; Yuan-quan, C.H.E.N.; Peng, S.U.I. 2022. Yield penalty of maize (Zea mays L .) under heat stress in different growth stages : A review. *J. Integr. Agric*. 2022, *21*(9), 2465-2476: doi: 10.1016/j.jia.2022.07.013.

Tesfaye, K.; Kruseman, G.; Cairns, J.E.; Zaman-Allah, M.; Wegary, D.; Zaidi, P.H.; Boote, K.J.; Erenstein, O. Potential benefits of drought and heat tolerance for adapting maize to climate change in tropical environments. *Clim. Risk Manag.* 2018, *19*, 106-119: doi: 10.1016/j.crm.2017.10.001.

Thayil, V.M.; Zaidi, P.H.; Seetharam, K.; Rani Das, R.; Viswanadh, S.; Ahmed, S.; Miah, M.A.; Koirala, K.B.; Tripathi, M.P.; Arshad, M.; Pandey, K. Genotype-by-environment interaction effects under heat stress in tropical maize. *Agronomy,* 2020, 10(12), 1998: doi: 10.3390/agronomy10121998.

Thompson, L.M. Weather variability and the need for a food reserve. 1966. https://dr.lib.iastate.edu/handle/20.500.12876/12208.

Tian, X.; Wang, F.; Zhao, Y.; Lan, T.; Yu, K.; Zhang, L.; Qin, Z.; Hu, Z.; Yao, Y.; Ni, Z.; Sun, Q. Heat shock transcription factor A1b regulates heat tolerance in wheat and Arabidopsis through OPR3 and jasmonate signalling pathway. *Plant Biotechnol. J.* 2020, *18*(5), 1109-1111: doi: 10.1111/pbi.13268.

Tiwari, Y.K.; Yadav, S.K. High temperature stress tolerance in maize (Zea mays L.): Physiological and molecular mechanisms. *J. Plant Biol.*, 2019, *62 (2)*, 93-102: doi: 10.1007/s12374-018-0350-x.

Trachsel, S.; Dhliwayo, T.; Gonzalez Perez, L.; Mendoza Lugo, J.A.; Trachsel, M. Estimation of physiological genomic estimated breeding values (PGEBV) combining full hyperspectral and marker data across environments for grain yield under combined heat and drought stress in tropical maize (Zea mays L.). *Plos one*, 2019. *14*(3), 1-15: https://doi.org/10.1371/journal.pone.0212200

Trapero-Mozos, A.; Morris, W.L.; Ducreux, L.J.; McLean, K.; Stephens, J.; Torrance, L.; Bryan, G.J. Hancock, R.D.; and Taylor, M.A. Engineering heat tolerance in potato by temperature-dependent expression of a specific allele of heat-shock cognate *Plant Biotechnol. J.* 2018. *16*(1), 197-207: doi: 10.1111/pbi.12760.

Trentin, H.U.; Frei, U.K.; Lübberstedt. T. Breeding maize maternal haploid inducers. Plants. 2020. 9(5). doi: 10.3390/plants9050614.

Tuberosa, R.; Salvi. S. Genomics-based approaches to improve drought tolerance of crops. Trends Plant Sci. 2006, 11(8), 405–412. doi: 10.1016/j.tplants.2006.06.003.

Uddin, M.S.; Amiruzzaman, M.; Bagum, S.A.; Hakim, M.A.; Ali, M.R. Combining ability and heterosis in maize (Zea mays L.). *Bangladesh J. Plant Breed. Genet.* 2008, *21*(1), 21-28: doi: 10.3329/bjpbg.v21i1.17045.

Valdés-López, O.; Batek, J.; Gomez-Hernandez, N.; Nguyen, C.T; Isidra-Arellano, M.C.; Zhang, N.

Joshi, T.; Xu, D.; Hixson, K.K.,; Weitz, K.K.; Aldrich, J.T. Soybean roots grown under heat stress show global changes in their transcriptional and proteomic profiles. *Front. Plant Sci.* 2016, *7*, 1-12: doi: 10.3389/fpls.2016.00517.

Wahid, A.; Gelani, S.; Ashraf, M.; Foolad, M.R. Heat tolerance in plants: an overview. *Environ. Exp. Bot.*, 2007, *61*(3), 199-223: doi: 10.1016/j.envexpbot.2007.05.011.

Wang, Q.L.; Chen, J.H.; He, N.Y.; Guo, F.Q. Metabolic reprogramming in chloroplasts under heat stress in plants. *Int. J. Mol. Sci.* 2018, *19*(3), 9-11: doi: 10.3390/ijms19030849.

Wang, W.; Vinocur, B.; Shoseyov, O.; Altman, A. Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. *Trends Plant Sci.* 2004. *9*(5), 244-252. doi: 10.1016/j.tplants.2004.03.006.

Waqas, M.A.; Wang, X. ; Zafar, S.A.; Noor, M.A.; Hussain, H.A.; Azher Nawaz, M.; Farooq, M. 2021. Thermal stresses in maize: effects and management strategies. *Plants*, *10*(2), 1-23: doi: 10.3390/plants10020293.

Waraich, E.A.; Ahmad, R.; Halim, A.; Aziz, T. Alleviation of temperature stress by nutrient management in crop plants: A review. *J. Soil Sci. Plant Nutr.* 2012. *12*(2), 221-244: doi: 10.4067/S0718-95162012000200003.

Worku, M.; Makumbi, D.; Beyene, Y.; Das, B.; Mugo, S.; Pixley, K.; Bänziger, M.; Owino, F.; Olsen, M.; Asea, G.; Prasanna, B.M. Grain yield performance and flowering synchrony of CIMMYT's tropical maize (Zea mays L.) parental inbred lines and single crosses. *Euphytica*, 2016, *211*(3), 395-409: doi: 10.1007/s10681-016-1758-3.

Yadav, O.P.; Hossain, F.; Karjagi, C.G.; Kumar, B.; Zaidi, P.H.; Jat, S.L.; Chaula,S.; Kaul, J.; Hooda, K.S.; Kumar, p.; Yadav, P.; Dhillon, B.S. Genetic Improvement of Maize in India: Retrospect and Prospects. *Agric Res* , 2015, (**4)** 325–338: https://doi.org/10.1007/s40003-015-0180-8

Yadav, S.K.; Tiwari, Y.K.; Pavan Kumar, D.; Shanker, A.K..; Jyothi Lakshmi, N.; Vanaja, M.; Maheswari, M. Genotypic variation in physiological traits under high temperature stress in maize. *Agric. Res. J.* 2016. *5*, 119-126: doi: 10.1007/s40003-015-0202-6.

Yadav, S.K.; Tiwari, Y.K.; Singh, V.; Patil, A.A.; Shanker, A.K.; Jyothi Lakshmi, N.; Vanaja, M.; Maheswari, M.. Physiological and biochemical basis of extended and sudden heat stress tolerance in maize. *Proc. Natl. Acad. Sci. India Sect. B - Biol. Sci.*2018, *88(1),* 249-263: doi: 10.1007/s40011-016-0752-9.

Yan, W.; Tinker, N.A. Biplot analysis of multi-environment trial data: Principles and applications. *Can. J. Plant Sci*. 2006, 86(3): 623–645: doi: 10.4141/P05-169.

Yang, Z.; Jiang, Y.; Qiu, R.; Gong, X.; Agathokleous, E.; Hu, W.; Clothier, B. Heat stress decreased transpiration but increased evapotranspiration in gerbera. *Front. Plant Sci.* 2023. *14*. 1119076. https://doi.org/10.3389/fpls.2023.1119076

Yano, K.; Yamamoto, E.; Aya, K.; Takeuchi, H.; Lo, P.C.; Hu, L.; Yamasaki, M.; Yoshida, S.; Kitano, H.; Hirano, K.; Matsuoka, M. Genome-wide association study using whole-genome sequencing rapidly identifies new genes influencing agronomic traits in rice. *Nat. Genet.* 2016, *48*(8), 927-934: doi: 10.1038/ng.3596.

Yousaf, M.I.; Hussain, K.; Hussain, S.; Ghani, A.; Arshad, M.; Mumtaz, A.; Hameed, R.A. Characterization of indigenous and exotic maize hybrids for grain yield and quality traits under heat stress. *Int. J. Agric. Biol.* 2018, *20*(2), 333-337: doi: 10.17957/IJAB/15.0493.

Yuan, Y.; Cairns, J. E.; Babu, R.; Gowda, M.; Makumbi, D.; Magorokosho, C.; Zhang, A.; Liu, Y.; Wang, N.; Hao, Z.et. al. Genome-wide association mapping and genomic prediction analyses reveal the genetic architecture of grain yield and flowering time under drought and heat stress conditions in maize. *Front. Plant Sci.* 2019, 9, 1-5: 1–15. doi: 10.3389/fpls.2018.01919.

Zaidi, P.H.; Nguyen, T.; Ha, D.N.; Thaitad, S.; Ahmed, S.; Arshad, M.; Koirala, K.B.; Rijal, T.R.; Kuchanur, P.H.; Patil, A.; Mandal, S.S. Stress-resilient maize for climate-vulnerable ecologies in the Asian tropics. *Aust. J. Crop Sci.* 2020.*14*(8), 1264-1274: doi: 10.21475/ajcs.20.14.08.p2405.

Zaidi, P.H.; Vinayan, M.T.; Nair, S.K.; Kuchanur, P.H.; Kumar, R.; Singh, S.B.; Tripathi, M.P.; Patil, A.; Ahmed, S.; Hussain, A.; Kulkarni, A.P. Heat-tolerant maize for rainfed hot, dry environments in the lowland tropics: From breeding to improved seed delivery. *Crop J.* 2023, *11*(4), 986-1000: doi: 10.1016/j.cj.2023.06.008.

Zhao, Y.; Hu, F.; Zhang, X.; Wei, Q.; Dong, J.; Bo, C.; Cheng, B.; Ma, Q. Comparative transcriptome analysis reveals important roles of non-additive genes in maize hybrid under heat stress. *BMC plant biol*, 2019, *19*, 1-17. doi: 10.1186/s12870-019-1878-8.

Zinn, K.E.; Tunc-Ozdemir, M.; Harper, J.F. Temperature stress and plant sexual reproduction: uncovering the weakest links. *J. Exp. Bot.* 2010, *61*(7), 1959-1968: doi: 10.1093/jxb/erq053.

Ziyomo, C.; Bernardo, R. Drought tolerance in maize: Indirect selection through secondary traits versus genomewide selection. *Crop Sci.* 2013, *53*(4), 1269-1275: doi: 10.2135/cropsci2012.11.0651.

CHAPTER 3

Genetic potential of tropically adapted exotic maize (Zea mays L.) heat-tolerant donor lines in sub-tropical breeding programmes

---

Abstract

Breeding for heat stress tolerance became a priority in sub-Saharan Africa (SSA), as projections are showing an increase in frequency, duration, and severity of high temperature. In this study, 14 heat stress tolerant-donor lines (HSTDLs) sourced from CIMMYT-India (males) were crossed with 15 locally adapted elite lines (females) developed within the CIMMYT-Zimbabwe maize-breeding programme using the North Carolina Design II mating scheme. The resultant 175 single crosses were evaluated alongside five commercial hybrids and adjacent to the trial of parental lines used in the crosses across two locations representing heat stress and optimal environments in Zimbabwe. The design II analysis showed significant ($p < 0.01$) general combining ability (GCA) effects for exotic heat donor lines and specific combining ability (SCA) effects on grain yield under heat stress, optimal conditions, and across locations; demonstrating additive and non-additive genetic inheritance of grain yield. High Baker's ratios observed in this study indicate predominance of additive over non-additive gene effects. Three exotic HSTDLs, namely CAL14138, CAL152, and CAL1440, exhibited significant ($p < 0.001$) and positive GCA effects under heat stress conditions. The results imply that these exotic lines could serve as valuable genetic resources for introgression of heat tolerant alleles into local maize populations for accelerated yield genetic gains. Single crosses, DJ265-15 × VL1018816 and DJ267-9 × CAL1440, exhibited positive and significant ($p < 0.01$) and ($p < 0.05$) SCA effects for grain yield under heat stress conditions, respectively. These crosses can be used for further breeding and can contribute to grain yield performance under heat stress conditions. The exotic HSTDLs, CAL14138, CAL152, and VL109126 showed superior *per se* performance under heat, optimal conditions, and across environments. Overall data demonstrate the potential of exotic HSTDLs for improving the adaptation of maize to heat stress in sub-tropical breeding programmes.

Keywords: combining ability; exotic germplasm; gene action; heat stress

3.1 Introduction

Over 650 million people in sub-Saharan Africa (SSA) depend on maize (*Zea mays* L.) for their livelihoods and for daily calorie intake [1]. However, despite the importance of maize in SSA, heat stress, which can occur at any growth stage, became an important abiotic stress constraining yield, especially during the flowering and early grain-filling stages. The stress induces anatomical, biochemical, morpho-physiological, and molecular changes in crops, resulting in significant grain yield losses [2]. During the pollination and grain filling stages, a temperature of 35 °C on maize caused a daily grain yield reduction of 101 kg ha^{-1} [3]. Moreover, maize grain yield losses of up to 42% and 70% due to heat stress were reported by Khodarahmpour et al. and Badu-Apraka et al. [4,5], respectively. Climate projections show that heat stress frequency, duration, and severity is increasing and that a temperature rise of 0.3 °C is expected to reach approximately 1 °C above the present value by the year 2025 [6,7], and this will cause a decline in global maize grain yield potential by 45% [8]. Hence, acceleration of breeding for heat stress tolerance became a priority objective in maize breeding to ensure food security under these predicted climatic scenarios [9].

Breeding for heat stress tolerance in tropical maize is in its infancy stage, as most programmes focus on developing maize cultivars that are tolerant to drought stress, poor soil fertility, diseases, and pests [4,10–12]. In Zimbabwe, no heat-tolerant maize cultivars are commercialized, despite most of the maize production taking place in heat stress-prone areas. It is, therefore, imperative to develop and release maize cultivars that are tolerant to heat stress. On the other hand, literature pertaining to gene action controlling important traits in maize under heat stress are limited [13–15], and this warrants considerable attention. In order to start an appropriate heat stress tolerance breeding programme, it is essential to have knowledge on genetic diversity for heat tolerance, gene action, and inheritance of the traits of interest [16]. Additionally, information on general combining ability (GCA) and specific combining ability (SCA) of the breeding population is a fundamental prerequisite for effective selection, genetic improvement, and adoption of the appropriate breeding strategy [17–19].

As part of its maize genetic improvement for stress adaptation agenda, the International Maize and Wheat Improvement Centre (CIMMYT)-Zimbabwe sourced heat-tolerant donors from CIMMYT-India. These lines are the products of targeted breeding for heat stress tolerance under the project 'Heat tolerant maize for Asia (HTMA)' funded by the United States Agency for International Development (USAID), wherein multi-parent populations were improved using genomic selection approach. The lines were extracted from the improved cycle (C3) of the populations using doubled haploid (DH) technology and test cross progenies evaluated across locations under natural heat stress conditions in South Asia, resulting in the identification of lines with high GCA for heat stress tolerance [1]. However, the genetic value of these heat-tolerant lines in SSA is not known. Therefore, to determine the breeding value of these lines for their utility in crop improvement, selection of parents based on their combining ability is important [20]. General and specific combining ability are, respectively, associated with additive gene action and non-additive gene action modulated by dominance and epistatic gene effects [19,20].

Combining ability for heat tolerance in maize was studied by several researchers, and both additive and non-additive gene action were reported on the expression of grain yield, anthesis-silking interval, leaf firing, tassel blasting, and anthesis dates [15,21,22]. Studies conducted by Jodage et al. and Chapman et al. [15,21] showed that additive genetic effects play a major role in conditioning grain yield under heat stress conditions in tropical maize. However, some researchers reported contrasting genetic effects for grain yield under heat stress conditions [14]. Hence, there is a need to conduct further research to have conclusive results. Therefore, the objectives of this study were to: (i) identify Indian HSTDLs that can positively and significantly contribute to grain yield performance in combination with CIMMYT-Zimbabwe elite lines under heat stress and optimal conditions, (ii) identify single crosses with significant positive SCA for grain yield that can be used in further breeding for heat stress in SSA, and (iii) determine the gene action that controls the inheritance of grain yield under heat stress. We hypothesize that exotic HSTDLs can significantly contribute to adaptation of maize under heat stress conditions in SSA.

3.2 Materials and Methods

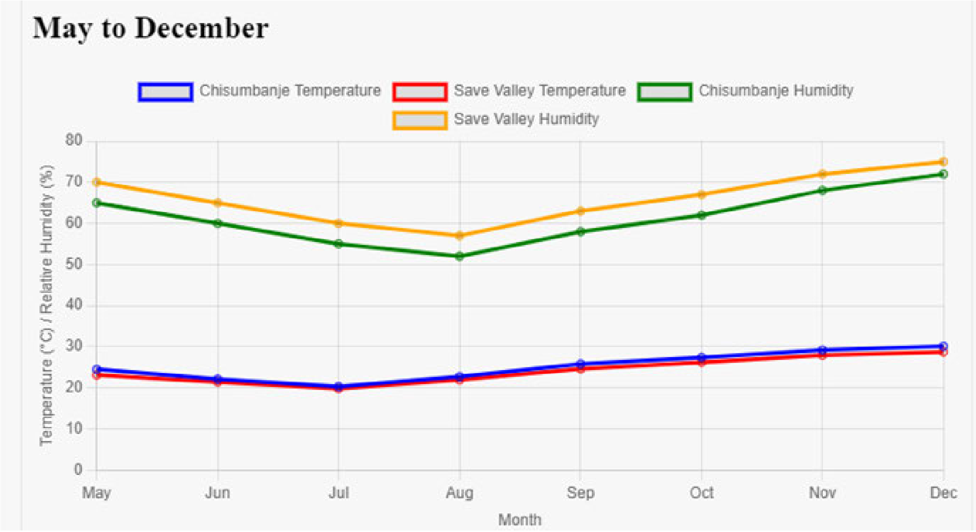
3.2.1 Germplasm

A total of 14 HSTDLs sourced from CIMMYT-India were crossed with 15 elite lines developed within the CIMMYT-Zimbabwe programme, using the North Carolina Design II mating scheme [23]. Out of the 210 crosses that were expected only 175 crosses were successful and had sufficient seed to conduct trial across four sites replicated two times. The exotic donor lines were selected for reduced anthesis-silking interval, good combining ability for yield under heat stress, and desirable agronomic traits (including standability, husk cover, and disease tolerance) [15], making them ideal donor lines for heat stress tolerance breeding in the sub-tropical maize breeding programmes. The exotic lines were used as males, while the elite CIMMYT-Zimbabwe lines were used as females. The crossing nursery was established at CIMMYT-Muzarabani station (altitude = 343 m above sea level (masl), GPS = −16°39′ S, 31°01′ E, soil type = red clay soils, average minimum winter temperature = 14 °C, and average maximum winter temperature = 28 °C) during the 2019 winter season and yielded 175 single crosses with sufficient seed to use in trials. The crosses were evaluated together with five commercial hybrids (CZH15429, SC633, SC608, SC727, and SC719) at two locations during the 2020 winter season, under two management regimes (i.e., managed heat stress and well-watered conditions) in the lowveld area of Zimbabwe (Table 3.1). The male and female parental lines, together with six check inbred lines, constituted the line trial (Table 3.2), which was planted side by side with the hybrid trials at all the locations.

Table 3.1 Description of experimental sites used in the evaluation of the single-cross hybrids and the parents.

Location	Management	Altitude (masl)	Latitude	Longitude	Soil Type
Chisumbanje	Managed heat stress and optimal	423	−20°47’97.10” S	32°14’05.0” E	Black clays
Save Valley	Managed heat stress and optimal	450	−20°51’94.9” S	33°15’93.3” E	Ferralsols

masl = Meters above sea level.



Long-term temperature and relative humidity of Chisumbanje and Save Valley experiment stations.

Table 3.2 Description of the local lines and the exotic heat tolerant donor lines inter-mated in design II scheme at the CIMMYT-Muzarabani station during the 2018 winter season.

Entry	Line Name	Heterotic Group	Grain Color	Germplasm Source
Local Lines				
FL1	DJ194-3	B	White	CIMMYT-Zimbabwe
FL2	DJ267-5	A	White	CIMMYT-Zimbabwe
FL3	DJ267-6	A	White	CIMMYT-Zimbabwe
FL4	DJ267-7	A	White	CIMMYT-Zimbabwe
FL5	DJ267-8	A	White	CIMMYT-Zimbabwe
FL6	DJ194-10	A	White	CIMMYT-Zimbabwe
FL7	DJ194-2	B	White	CIMMYT-Zimbabwe
FL8	DJ267-9	A	White	CIMMYT-Zimbabwe
FL9	DJ265-6	B	White	CIMMYT-Zimbabwe
FL10	DJ265-8	A	White	CIMMYT-Zimbabwe
FL11	DJ265-10	A	White	CIMMYT-Zimbabwe
FL12	DJ265-15	A	White	CIMMYT-Zimbabwe
FL13	DJ265-13	B	White	CIMMYT-Zimbabwe
FL14	DJ265-14	B	White	CIMMYT-Zimbabwe
FL15	DJ265-15	B	White	CIMMYT-Zimbabwe
Exotic lines				
ML1	CAL14113	B	Yellow	CIMMYT-India
ML2	CAL1412	A	Yellow	CIMMYT-India
ML3	CAL14135	B	Yellow	CIMMYT-India
ML4	CAL14138	A	Yellow	CIMMYT-India
ML5	CAL1440	A	Yellow	CIMMYT-India
ML6	CAL1469	A	Yellow	CIMMYT-India
ML7	CAL152	A	Yellow	CIMMYT-India
ML8	VL1010762	A	Yellow	CIMMYT-India
ML9	VL1018816	B	Yellow	CIMMYT-India
ML10	VL109126	A	Yellow	CIMMYT-India
ML11	VL143518	B	Yellow	CIMMYT-India
ML12	ZL111056	A	Yellow	CIMMYT-India
ML13	ZL1312	B	Yellow	CIMMYT-India
ML14	ZL132077	A	Yellow	CIMMYT-India

FL—Female line, ML—male line.

3.2.2 Experimental design and trial management

The hybrid trial (175 single crosses + 5 commercial check hybrids) was laid out in the field using an alpha (0.1) lattice design and replicated two times with 36 incomplete blocks nested in each replicate with a block size of five. On the other hand, the line trial, which consisted of 35 lines (14 exotic + 15 local elite + 6 checks), was also laid out in the field using alpha (0.1) lattice design and replicated twice, with 7 incomplete blocks nested within each replicate, with a block size of five. Each entry was planted in a single-row plot measuring 4 m in length, with an in-row spacing of 0.25 m and inter-row spacing of 0.75 m. Two seeds were planted per each planting station and thinned to one plant/station two weeks after germination to give a plant population of approximately 53,000 plants ha^{-1}.

Heat stress was induced by adjusting planting dates so that the flowering period coincided with the hottest period (October–November), and this was achieved by establishing the trials in the last week of July, 2020. Trials under optimal management were planted earlier in May and were supplied with irrigation water whenever the need arose. All trials received basal fertilizer (compound D: 7% N, 14% P, and 7% K), which was applied at a rate of 300 kg ha^{-1}. Top dressing was split and applied at a rate of 150 kg ha^{-1} AN (ammonium nitrate) per application at four and eight weeks after crop emergence. Karate (*Lambdacyhalomethrin*) was applied at a rate of 100 mL per hectare before sowing to control ants and termites. Emamectin benzoate and acetamiprid were applied to the crop at a rate of 250 mL ha^{-1} to control fall armyworm *(Spodoptera frugiperda*) and maize stalk borer (*Busseola fusca)*. Hand weeding was the predominant form of weed control at both sites; however, selective application of paraquat at the rate of 1.5 l ha^{-1} was conducted at both sites between 10 and 11 weeks.

3.2.3 Data collection

In all the trials, data for flowering dates (days to 50% anthesis and 50% silking), plant height (PH; cm), ear height (EH; cm), and yield (GYD; kg) were recorded (Table 3.3). Some derived traits such as anthesis-silking interval (ASI) and grain yield (12.5% moisture adjustment) were calculated. Weather data (i.e. maximum and minimum temperature (°C) and relative humidity (%)) were measured using the Davis automatic weather station (Table 3.4). The weather readings were measured using sensors attached to the station through wireless connection.

Table 3.3 Description of traits recorded in the study.

Trait	Trait Measurement
Grain yield (GYD)	Shelled grain weight (kg) per plot adjusted to 12.5% grain moisture.
Plant height (PH)	Distance (cm) of two average plants measured from the ground up to the flag leaf collar.
Ear height (EH)	Distance (cm) of two average plants measured from the ground up to the ear height.
Silking date (SD)	Number of days after planting when 50% of the plants in each plot produce silks
Anthesis date (AD)	Number of days after planting when 50% of the plants in each plot shed pollen.
Anthesis-silking interval (ASI)	Difference between the silking date and anthesis date ASI = SD-AD.

Table 3.4 Temperatures and relative humidity measured during 2020 winter season.

	Chisumbanje Experiment Station							**Save Valley Experiment Station**						
Temp (°C)	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**
Max	27.0	23.8	23.7	28.2	30.0	31.2	34.1	29.7	26.4	26.3	30.4	32.1	34.6	36.3
Min	13.9	12.2	10.9	13.0	15.4	16.5	20.1	15.8	13.9	12.5	15.0	17.1	19.3	21.7
R.H (%)	59.0	65.9	60.3	53.4	55.2	55.7	57.4	52.1	59.7	54.1	50.5	51.5	52.7	56.2

Aug = August, Sept = September, Oct = October, Nov = November, Max= maximum temperature, Min = minimum temperature, Temp = temperature, and RH = relative humidity.

3.2.4 Data Analysis

Analysis of variance (ANOVA) for the grain yield within and across environments was performed using restricted maximum likelihood (REML) and the linear mixed models in the Genstat Discovery Software V20.0 [24]. In the analysis, entries were regarded as fixed factors, whilst sites, replications, and incomplete blocks were considered random factors. The Wald statistic ($p < 0.05$) was used to test the significance of various ANOVA components. Bartlett's chi-square test was used to test the homogeneity of residual variances between the environments before combined analysis was performed. Because the Bartlett's test revealed heterogeneity of residual variance for grain yield, square root transformation was employed to improve normality of the residuals [25].The ANOVA for a single site was computed according to the linear model by Barreto et al. [26] as follows:

$$Y_{ijk} = \mu + r_i B_k + G_j + \varepsilon_{ijk} \qquad (1)$$

where Y_{ijk} is the response variable, μ is the grand mean, r_i is the random effect of the ith replication, B_K is the effect of the k^{th} genotype nested in the i^{th} replication, G_j is the effect of the j^{th} genotype and εijk is the residual random error associated with the observation Yijk

A combined ANOVA was also carried out according to the linear model by Barreto et al. [26] as follows:

$$Y_{ijkl} = \mu + r_j B_k + L_i + Gl + GL_{il} + \varepsilon_{ijkl} \qquad (2)$$

where Y_{ijkl} is the response variable, μ is the grand mean, $r_j B_K$ is the effect of the k^{th} block nested in the j^{th} replication, , L_i is the effect of i^{th} location, G_l is the effect of the l^{th} entry and , GL_{il} is the interaction effect of the l^{th} genotype and the i^{th} location, and ε_{ijkl} is the experimental error.

Best linear unbiased predictions (BLUPs) of the parents and hybrids were computed using the Multi-Environment Trials Analysis in R (META-R) V2.1 R package software [27] and mean separation was conducted using Fisher's unprotected least significant differences (LSD) procedure at a 0.05 probability level. The relative importance of general (GCA) and specific (SCA) combining ability effects were calculated as a proportion of the cross effect sum of squares using the North Carolina Design II analysis model in the Analysis of Genetic Designs in R (AGD-R) V3.0 R software [28]. The combining ability analysis was conducted on 169 hybrids, which were generated by crossing 13 males (heat tolerant donors) and 13 female (locally adapted lines). Six experimental hybrids and 5 check varieties were excluded from the analysis.

The relative importance of additive and non-additive gene action in the expression of the grain yield under heat stress, optimal conditions, and across environments, was estimated using Baker's ratio [29], which was calculated from the GCA and SCA variance components following Falconer [30],

$$\text{Baker's ratio} = (\sigma^2\, GCA_f + \sigma^2\, GCA_m) / (\sigma^2\, GCA_f + \sigma^2 GCA_m + \sigma^2\, SCA) \tag{3}$$

where $\sigma^2\, GCA_f$ is the GCA female variance, $\sigma^2\, GCA_m$ is the GCA male variance, and σ^2 SCA is the SCA variance. If the Baker's ratio is >0.5, it implies that GCA is more important than SCA in the inheritance of the character, and a ratio < 0.5 implies that SCA is more important than GCA in the inheritance of the character [29].

From the factorial mating design, narrow sense heritability (h^2) was determined based on female additive variance following Dabholkar [31],

$$h^2_f = 4\sigma^2 GCA_f/(\sigma^2 e/r + 4\sigma^2 SCA + \sigma^2 GCA_f) \tag{4}$$

where h^2_f is the female narrow sense heritability based on GCA and SCA variances; $\sigma^2 GCA_f$, is variance for general combining ability of female parents; σ^2SCA is the variance of specific combining ability; σ^2e is the error variance; and r is number of replications.

Heritability was computed based on the female additive variance because from a balanced factorial design, the magnitude of SCA and GCA are equal, and if not, then there should be a maternal effect. Therefore, female additive variance was preferred to males because it accounts for any maternal effects in the cross [31]. Broad-sense heritability (H^2) was calculated following Dabholkar [31] as follows:

$$H^2 = 4\sigma^2 GCA_f + 4\sigma^2 SCA/(\sigma^2 e/r + 4\sigma^2 SCA + \sigma^2 GCA_f). \tag{5}$$

3.3 Results

3.3.1 Performance of the hybrids and the parents under heat stress and non-Stress Conditions

Combined analysis of variance (ANOVA) showed significant ($p < 0.05$) effects for grain yield under managed heat stress, optimal conditions, and across environments (Table 5). The general combining ability–female (GCAf) and specific combining ability (SCA) mean squares also showed significant ($p < 0.001$) effects for grain yield under managed heat stress, optimal conditions, and across environments (Table 5). Similarly, the general combining ability–male (GCAm) mean squares showed significant ($p < 0.01$) effects for grain yield under managed heat stress, optimal conditions, and across environments. The genotype by environment interaction (GEI) mean squares for $GCA_{f,}$ $GCA_{m,}$ and SCA were significant ($p< 0.001$) for grain yield across environments. Additive gene action appeared to be more important than dominance gene action under both heat stress and optimal conditions (Table 3.5).

Table 3.5 Analysis of variance, variance components and heritability for grain yield performance of the North Carolina Design II crosses evaluated during the 2020 winter season in Zimbabwe.

	Managed Heat Stress		Optimal Management		Across	
Source of variation	DF	MS	DF	MS	DF	MS
Site	1	48.02 ***	1	501.71 ***	3	437.49 ***
Rep (site)	2	50.68 ***	2	7.98 **	4	29.35 ***
Block (rep × site)	140	0.67 ^{ns}	140	2.47 **	280	1.78 ***
Female	12	2.44 ***	12	9.41 ***	12	7.82 ***
Male	12	1.97 **	12	8.47 ***	12	7.72 ***
Female × male	144	0.78 ^{ns}	182	3.61 *	144	2.85 ***
Female × site	12	0.83 ^{ns}	12	7.49 ***	36	3.7 ***
Male × site	12	2.10 ***	13	6.82 ***	36	6.12 ***
Female ×male × site	144	0.84 ^{ns}	144	2.6 **	432	1.93 ***
Male variance		0.596		0.744		0.667
Female variance		0.008		0.054		0.007
Male × female variance		0.287		0.808		0.405
Genotype variance		0.871		1.581		1.061
Additive variance (AV)		3.486		6.325		4.244
Dominance variance (DV)		1.149		3.234		1.620

Environmental variance (EV)	0.473	1.704	0.631
Baker’s ratio	0.68	0.50	0.62
Narrow sense heritability (h^2_f)	0.023	0.052	0.01
Broad-sense heritability (H^2)	0.85	0.83	0.85

*, **, and *** are significant at the 0.05, 0.01, and 0.001 probability levels, respectively, ns = non-significant, DF = degrees of freedom, MS = mean squares, and Rep = replication.

The male variance was generally higher than the female variance across all the test environments. Similarly, genotypic variances were larger than male-by-female variances for grain yield across environments. Additive variances were larger than dominance variances under managed heat stress, optimal conditions, and across environments. Both the additive and dominance variances were larger than the environmental variance. Variation due to the environment was higher than genotypic variation under all the environments used (Table 3.5).

The broad-sense heritability (H^2) for grain yield was higher than narrow sense heritability (h^2) across all the environments. Broad-sense heritability estimates for grain yield were greater than 80% across environments. Baker’s ratio estimates for grain yield were 0.68, 0.5, and 0.62 under heat stress, optimal conditions, and across environments, respectively.

3.3.2 Exotic donor lines that confer heat tolerance attributes in combination with the locally adapted parental lines

The best exotic HSTDLs with positive GCA effects for grain yield and high *per se* performance (PSP) values for grain yield under managed heat stress were identified as: CAL14138 (GCA = 1.89, PSP = 1.84 t ha^{-1}), CAL152 (GCA = 1.02, PSP = 1.71 t ha^{-1}), and CAL1440 (GCA = 0.83, PSP = 1.23 t ha^{-1}) (Table 6). Under optimal conditions, the exotic HSTDLs: CAL14138 (GCA = 1.84 ^{1}, PSP = 1.6 t ha^{-1}), CAL152 (GCA = 0.95, PSP = 2.09 t ha^{-1}), and CAL1440 (GCA = 0.99, PSP = 1.76 t ha^{-1}) had the highest GCA effects for grain yield coupled with high PSP for grain yield. Across environments, the exotic HSTDLs: CAL14138 (GCA = 1.92, PSP = 1.72 t ha^{-1}), CAL1440 (GCA = 0.94; PSP = 1.41 t ha^{-1}), and CAL152 (GCA = 1.02; PSP = 1.92 t ha^{-1}) had the highest positive GCA effects as well as PSP for grain yield. It is interesting to note that the exotic HSTDLs CAL14138, CAL152, and CAL1440, all showed significant and positive GCA effects for grain yield under managed heat stress, optimal conditions, and across environments, which was in every case, coupled with superior *per se* performance grain yield (Table 3.6).

Table 3.6 Estimates of the general combining ability and heritability of heat-tolerant donor lines and their *per se* grain yield performance in North Carolina Design II crosses and line trials conducted in Zimbabwe during the 2020 winter season.

Line	Exotic Donors	Managed Heat			Optimal Management			Across Environments		
		PSP	GCA	Rank GCA	PSP	GCA	Rank GCA	PSP	GCA	Rank GCA
A. Exotic HSTDLs (males)										
ML1	CAL14113	0.43	−0.374 ^{ns}	8	0.60	−0.597 ^{ns}	12	0.51	−0.500 *	10
ML2	CAL1412	0.88	−0.761 **	14	1.28	−0.585 ^{ns}	10	1.08	−0.709 *	13
ML3	CAL14135	0.58	−0.385 ^{ns}	10	0.58	−0.981 *	14	0.58	−0.723 **	14
ML4	CAL14138	1.84	1.889 ***	1	1.60	1.840 ***	1	1.72	1.918 ***	1
ML5	CAL1440	1.23	0.826 ***	3	1.76	0.990 **	2	1.41	0.942 ***	3
ML6	CAL1469	0.52	−0.235 ^{ns}	6	0.53	0.450 ^{ns}	4	0.53	0.134 ^{ns}	5
ML7	CAL152	1.71	1.017 ***	2	2.09	0.952 *	3	1.92	1.022 ***	2
ML8	VL1010762	0.33	−0.376 ^{ns}	9	0.55	−0.650 ^{ns}	13	0.44	−0.531 *	11
ML9	VL1018816	0.94	−0.186 ^{ns}	5	0.91	−0.069 ^{ns}	6	0.93	−0.124 ^{ns}	6
ML10	VL109126	1.57	0.275 ^{ns}	4	2.22	0.016 ^{ns}	5	1.89	0.147 ^{ns}	4
ML11	VL143518	1.33	−0.541 *	13	1.27	−0.415 ^{ns}	9	1.30	−0.491 ^{ns}	9
ML12	ZL111056	0.39	−0.247 ^{ns}	7	0.58	−0.107 ^{ns}	7	0.47	−0.176 ^{ns}	7
ML13	ZL1312	0.71	−0.428 ^{ns}	11	0.98	−0.251 ^{ns}	8	0.87	−0.357 ^{ns}	8
ML14	ZL132077	1.08	−0.474 *	12	0.62	−0.593 ^{ns}	11	0.85	−0.553 *	12
B. CIMMYT-Zimbabwe elite lines (females)										
FL1	DJ194-3	0.68	−0.014	12	1.14	−0.068	11	0.90	−0.014	12
FL2	DJ267-5	0.31	0.044	2	1.04	−0.042	10	0.64	0.005	6
FL3	DJ267-6	1.77	−0.001	8	2.43	−0.124	14	2.08	−0.019	13
FL4	DJ267-7	0.71	0.012	5	0.72	0.02	4	0.71	0.007	5
FL5	DJ267-8	1.34	−0.045	14	1.23	0.013	6	1.27	−0.011	10
FL6	DJ194-10	0.88	−0.008	11	0.90	−0.039	8	0.88	−0.008	9
FL7	DJ194-2	0.79	0.023	4	0.59	0.007	7	0.68	0.008	4
FL8	DJ267-9	1.76	0.066	1	2.27	0.251	1	2.02	0.058	1
FL9	DJ265-6	1.20	−0.022	13	1.04	0.014	5	1.09	−0.004	8
FL10	DJ265-8	1.67	0.025	3	1.74	−0.04	9	1.72	0.001	7
FL11	DJ265-10	1.81	−0.003	9	1.94	0.226	2	1.89	0.035	2
FL12	DJ265-12	0.60	−0.003	10	1.03	−0.083	12	0.81	−0.014	11
FL13	DJ265-13	0.67	0.003	7	0.33	−0.152	15	0.48	−0.023	14
FL14	DJ265-14	1.95	0.010 ^{ns}	6	2.22	0.131	3	2.08	0.023	3
FL15	DJ265-15	0.65	−0.087 ^{ns}	15	0.73	−0.115	13	0.69	−0.044	15
Grand mean		1.05			1.23			1.14		
LSD (0.05)		0.74			0.77			0.77		
Heritability		0.27			0.57			0.77		

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns = non-significant, PSP = per se performance; GCA = general combining ability; and GYD = grain yield.

3.3.3 Exotic HSTDLs that can positively contribute to grain yield performance in single-cross hybrid combinations with local elite lines under managed heat stress and optimal conditions

Tables 3.7 and Appendix 3.1 show the estimates of SCA for grain yield performance of the exotic HSTDLs and CIMMYT-Zimbabwe elite line crosses under managed heat stress, optimal conditions, and across environments. The crosses with positive and significant SCA effects for grain yield under managed heat stress were identified as DJ265-15 × VL1018816 (SCA = 1.05 t ha^{-1}, Mean grain yield performance of the cross (MGYP$_{\text{cross}}$) = 5.36 t ha^{-1}) and DJ267-9 × CAL1440 (SCA = 0.73 t ha^{-1}; MGYP$_{\text{cross}}$ = 5.65 t ha^{-1}). Under optimal conditions, the best specific combiners were: DJ26-13 × CAL1469 (SCA = 1.49 t ha^{-1}; MGYP$_{\text{cross}}$ = 7.61 t ha^{-1}), DJ265-10 × VL109126 (SCA = 1.42 t ha^{-1}; MGYP$_{\text{cross}}$ = 7.56 t ha^{-1}), DJ267-8 × CAL1440 (SCA = 1.35 t ha^{-1}, MGYP$_{\text{cross}}$ = 8.08 t ha^{-1}), and DJ265-10 × CAL14138 (SCA = 1.33 t ha^{-1}, MGYP$_{\text{cross}}$ = 8.49 t ha^{-1}). Genotypes DJ265-10 × CAL14138 (SCA = 0.89 t ha^{-1}, MGYP$_{\text{cross}}$ = 8.49 t ha^{-1}) and DJ265-10 × VL109126 (SCA = 0.88 t ha^{-1}, MGYP$_{\text{cross}}$ = 7.56 t ha^{-1}) were the best specific combiners across environments.

3.3.4 *Per se* performance of exotic HSTDLs and the CIMMYT-Zimbabwe elite lines under stress and non-stress conditions

The *per se* performance of the CIMMYT-Zimbabwe and exotic HSTDLs with respect to grain yield, days to 50% anthesis (AD), and anthesis-silking interval (ASI) are presented in Table 3.8. The three best performing exotic HSTDLs in terms of grain yield under managed heat stress were identified as CAL14138 (1.84 t ha^{-1}), CAL152 (1.71 t ha^{-1}), and VL109126 (1.5 t ha^{-1}). Under optimal conditions, the best three yielders among the exotic HSTDLs were VL109126 (2.22 t ha^{-1}), CAL152 (2.09 t ha^{-1}), and CAL1440 (1.76 t ha^{-1}). Across environments, the best exotic HSTDLs yielders were CAL152 (1.92 t ha^{-1}), VL109126 (1.89 t ha^{-1}), and CAL14138 (1.72 t ha^{-1}). Interestingly, the exotic lines CAL152 and VL109126 showed superior performance under both stress and optimal conditions. The number of days to 50% anthesis ranged from 69 to 78, 72 to 80, and 71 to 79 under managed heat stress, optimal, and across environments, respectively. On the other hand, ASI ranged from −0.5 to 3.25, −0.75 to 4.25, and −0.87 to 3.73 under managed heat stress, optimal, and across environments, respectively. In addition, the number of days to 50% anthesis and ASI were shortened under managed heat stress conditions. The broad-sense heritability estimates of 27%, 57%, and 77% were observed for grain yield under managed heat stress, optimal, and across environments, respectively. The number of days to 50% anthesis was highly heritable under both managed heat stress and optimal conditions (Table 8).

Table 3.7 Best specific combiners for grain yield performance among the exotic HSTDLs and CIMMYT-Zimbabwe crosses evaluated under heat stress and optimal conditions during the 2020 winter season in Zimbabwe.

	Managed Heat		**Optimal Management**		**Across Environments**	
Hybrid	**MGYP (tha^{-1})/Rank**	**SCA Effect**	**MGYP (tha^{-1})/Rank**	**SCA Effect**	**MGYP (tha^{-1})/Rank**	**SCA Effect**
DJ265-15 × VL1018816	5.36 (12)	1.05 **	5.56 (77)	0.03	5.46 (35)	0.526
DJ267-9 × CAL1440	5.65 (7)	0.73 *	7.28(9)	0.7	6.62 (5)	0.739
DJ265-15 × CAL14138	6.45 (1)	0.697	6.19 (40)	−0.21	6.40 (10)	0.199
DJ265-14 ×ZL1312	4.23 (58)	0.673	6.48 (27)	0.93	5.64 (29)	0.857
DJ194-3 × CAL1412	4.21 (64)	0.624	5.33 (95)	0.3	4.76 (81)	0.492
DJ267-7×VL1010762	3.10 (146)	0.603	5.52 (83)	−0.23	4.42 (114)	0.174
DJ267-7×VL143518	4.37 (50)	0.6	5.65 (69)	0.39	5.04 (60)	0.516
DJ265-15 × CAL14113	4.40 (44)	0.541	6.23 (39)	1	5.42 (37)	0.825
DJ265-8 × VL1010762	4.46 (40)	0.54	5.96 (50)	0.63	5.27 (45)	0.624
DJ267-5 × CAL1440	5.40 (10)	0.533	5.40 (90)	−0.43	5.40 (39)	−0.003
Heritability	80%		66%		80%	
Grand Mean	3.95		5.43		4.7	
LSD	0.83		1.47		0.96	
CV	21.76		24.35		23.96	

*, ** are significant at the 0.05 and 0.01 probability levels, respectively, LSD = least significant differences, CV = coefficient of variation, SCA = specific combining ability, and MGYP = mean grain yield.

Table 3.8 Agronomic performance of the CIMMYT-Zimbabwe elite lines and the exotic HSTDLs evaluated under heat stress and optimal conditions during the 2020 winter season in Zimbabwe.

Exotic	Lines	Managed Heat Stress			Optimum Management			Across Environments		
		GY (t ha^{-1})	AD (days)	ASI (days)	GY (t ha^{-1})	AD (days)	ASI (days)	GY (t ha^{-1})	AD (days)	ASI (days)
A. Exotic HSTDLs										
ML4	CAL14138	1.84	73	0.50	1.60	74	1	1.72	74	0.37
ML7	CAL152	1.71	73	−0.50	2.09	75	0.75	1.92	74	−0.25
ML10	VL109126	1.57	69	0.52	2.22	72	1.5	1.89	71	0.62
ML11	VL143518	1.33	77	0.75	1.27	80	4.25	1.30	78	0.87
ML5	CAL1440	1.23	75	0	1.76	77	0.5	1.41	76	1.50
ML14	ZL132077	1.08	79	−0.74	0.62	78	0.25	0.85	79	−0.87
ML9	VL1018816	0.94	79	−0.01	0.91	80	0	0.93	79	−0.13
ML2	CAL1412	0.88	71	1.26	1.28	74	0.5	1.08	72	1.50
ML13	ZL1312	0.71	73	−0.01	0.98	73	0.75	0.87	73	0.75
ML3	CAL14135	0.58	75	0.75	0.58	79	−0.75	0.58	77	0.62
ML6	CAL1469	0.52	74	0	0.53	75	1.5	0.53	74	−0.12
ML1	CAL14113	0.43	77	−0.25	0.60	79	1	0.51	78	0
ML12	ZL111056	0.39	74	1.50	0.58	75	−0.75	0.47	75	1.50
ML8	VL1010762	0.33	77	−0.01	0.55	77	2.75	0.44	77	0.25
B. CIMMYT-Zimbabwe elite lines										
FL14	DJ265-14	1.945	74	−1.26	2.22	77	−0.25	2.08	75	−0.88
FL11	DJ265-10	1.81	76	1.76	1.94	79	−1	1.89	77	1.37
FL3	DJ267-6	1.77	76	3.23	2.43	77	1	2.08	76	3.73
FL8	DJ267-9	1.76	74	1.75	2.27	76	−0.25	2.02	75	1.88
FL10	DJ265-8	1.67	78	0	1.74	79	3	1.72	78	0.38
FL5	DJ267-8	1.34	77	0.50	1.23	79	1.5	1.27	78	0.37
FL9	DJ265-6	1.20	78	0.33	1.04	81	1.75	1.09	79	0.28
FL6	DJ194-10	0.88	78	0.75	0.90	79	−0.5	0.88	79	0.62
FL7	DJ194-2	0.79	73	1.00	0.59	76	0.75	0.68	75	0.12
FL4	DJ267-7	0.71	77	0.50	0.72	78	0.25	0.71	78	0.50
FL1	DJ194-3	0.68	75	1.24	1.14	76	1	0.90	75	1.12

Exotic	Lines	Managed Heat Stress			Optimum Management			Across Environments		
		GY (t ha^{-1})	**AD (days)**	**ASI (days)**	**GY (t ha^{-1})**	**AD (days)**	**ASI (days)**	**GY (t ha^{-1})**	**AD (days)**	**ASI (days)**
FL13	DJ265-13	0.67	77	−1.25	0.33	78	0.75	0.48	77	−0.76
FL15	DJ265-15	0.649	75	1.75	0.73	76	−0.25	0.69	75	1.25
FL12	DJ265-12	0.60	76	1.25	1.03	75	1.75	0.81	76	1.37
FL2	DJ267-5	0.31	77	−0.01	1.04	78	0.75	0.64	77	0.88
G.variance		0.06 ***	4.67 ***	0.42 **	0.18 ***	4.55 ***	0.23 $^{\text{ns}}$	0.19 ***	5.26 ***	0.55 ***
G × E variance		0.29 ***	0.56 $^{\text{ns}}$	0.17 $^{\text{ns}}$	0.19 ***	2.26 **	0.57 $^{\text{ns}}$	0.16 ***	0.75 **	0.15 $^{\text{ns}}$
Heritability		0.27	0.80	0.47	0.57	0.67	0.18	0.77	0.88	0.63
Grand mean		1.05	75.44	0.45	1.23	77.16	0.75	1.14	76.31	0.6
LSD (0.05)		0.74	3.72	2.41	0.77	4.11	3.39	0.77	3.92	2.99
CV (%)		35.65	2.52	274.36	31.79	2.72	230.67	34.27	2.62	255.33

, and * are significant at the 0.01, and 0.001 probability levels, respectively, ns = non-significant, GY = grain yield, AD = anthesis date, ASI = anthesis-silking interval, CV = coefficient of variation, LSD = least significant difference, G.variance = genotypic variance, and GxE variance = genotype by environment variance.

3.4 Discussion

Availability of sufficient genetic variability is a key component for effective selection in any breeding programme. In the absence of sufficient genetic diversity, exotic germplasm can be sourced and introduced to the programme to introgress the required genes [32,33]. Generally, breeders conduct combining ability studies before using exotic germplasm in their breeding programmes to identify good parents for the development of hybrids and superior parents to use in breeding cycles [16,34]. The selection of good parents for hybrid development is based on the performance of the parents in hybrid combination [20], while selection of superior parents for use in the breeding cycle is based on the *per ser* performance of parents [35].

In the current study, a set of heat-tolerant exotic donor lines from CIMMYT-India and elite lines from CIMMYT-Zimbabwe were evaluated for their combining ability effects and *per se* performance under managed heat stress and optimal conditions. The results of this study reveal significant GCA and SCA effects under managed heat stress, optimal, and across locations. This reflects the presence of wide genetic variability for heat stress tolerance among the inbred lines and their single-cross hybrids, providing an opportunity for selection. These findings suggest that both additive and non-additive genetic effects are important for the inheritance of grain yield. Therefore, hybridization and recurrent selection might be helpful for genetic enhancement of heat tolerance and superior performance under heat stress conditions [20]. The findings concur with several authors [17,19,22,36–38] who reported significant GCA and SCA for grain yield under heat-stressed and optimal conditions.

The significant GCA_f and GCA_m effects observed for grain yield under managed heat stress and optimal conditions imply that both parents contributed to the inheritance of grain yield in the hybrids under these conditions. The significant GCA_m × E, GCA_f × E, and SCA × E effects on grain yield performance observed under optimal conditions, show that the optimal sites were different from each other, an indication that the combining ability of the parental lines was not consistent under optimal conditions and the environment had an effect on grain yield. This suggests the need for selecting different parental lines for hybrids to be used in specific environments. Additionally, these results suggest that both additive and non-additive genetic effects interacted with the environment in the expression of grain yield, thus justifying a multi-location improvement strategy in the development of lines with tolerance to heat stress.

The non-significant GCA_m × E, GCA_f ×E, and SCA × E effects observed on grain yield performance under managed heat stress conditions reflect that the heat stress sites were comparable, and the inbred lines performed consistently across heat stress conditions; hence, the environment had no effect on the grain yield. These observations were inconsistent with the previous study by Osuman et al. [19], who reported significant interactions for grain yield under heat stress conditions. The findings also contradict with those of Derera et al. [39], who reported significant GCA_m, GCA_f, and SCA by environment interactions for grain yield under stress environments. The absence of significant SCA × E interaction for grain yield under heat stress environments suggested that grain yield would be stable in specific hybrid combinations under heat stress conditions. These results are consistent with those found by Ifie et al. and Oyekunle et al. [40,41], who reported lack of significant SCA × E interaction variances for grain yield under stress environments.

The prevalence of additive gene effects over dominance revealed by this study suggests that additive gene action was more important in the inheritance of grain yield under heat stress, optimal conditions, and across locations for the germplasm tested. These results are in line with the findings of Begna and Fasahat [20,34], who reported that when GCA effects prevail over SCA effects, early generation testing of crosses can be effectively conducted, and promising hybrids can be identified and selected based on the GCA effects of the crosses. However, using the GCA effects of the parents alone to predict hybrid performance will not always be sufficient because of the significant SCA [39]. This also implies that heat-tolerant inbred lines with high GCA effects could be extracted from improved cycles of selection of derived populations for hybrid development [42]. Additionally, this observation has the implication that strategies for population improvement that make use of additive gene action, such as S1 family selection, full-sib family, and half-sib family procedures, can be conducted to advance the development of superior hybrids for heat stress-prone environments. The high estimates of Baker's ratio (>0.5) observed on grain yield under all the test environments confirm the importance of additive genetic effects in the inheritance of grain yield, which implies that the genes controlling grain yield can easily be fixed in advanced generations. The presence of additive gene effects indicates a high chance for predicting the performance of progenies based on the phenotypic scores of the parents [43].

Predominance of additive gene action over the non-additive gene action was revealed by several authors under stress conditions in maize [19,38,39,44–46]. Because additive genetic variance is heritable and genotype is controlled, it affects the parent's breeding value [32]. Hence, the substantial additive variance over dominant variance observed in this study pointed out that the parents used were of high breeding value, allowing for rapid genetic improvement in the production of desired high yielding heat-tolerant genotypes.

The differences in heritability values for grain yield observed under stress, optimal conditions, and across environments were in line with the reports of several researchers [11, 22, 47]. The higher value of broad-sense heritability for yield, under heat stress, optimal conditions, and across environments suggests that environmental effects had a small role in the variation observed [48]. This result points to less of a need for using many locations and replications in this type of trial. This also reflects the genetic potential of the parents to largely pass on the grain yield trait to subsequent generations.

The GCA effects of an inbred line provide important information for the improvement of a target trait in a population [40]. Significant positive GCA effects for grain yield exhibited by heat donor lines CAL14138, CAL152, and CAL1440 in this study under heat stress, non-stress, and across environments reflect the high potential of these lines to transfer desirable traits to their offspring [38]. Therefore, these lines could be utilized in maize improvement programmes as sources of favorable alleles for the genetic enhancement of grain yield under heat stress [22]. Additionally, this result implies that the favorable alleles for grain yield could be easily introgressed into local material and hence improve heat stress tolerance in local material. These results are in agreement with those reported by Nasser et al. [38].

High GCA indicates the inherent genetic value of a parent due to the presence of additive genetic effects. Therefore, the heat-tolerant donors that exhibited positive significant GCA could produce superior segregates in the F2 and later generations, as they could serve as vital sources of beneficial alleles [49]. Furthermore, the presence of high GCA effects for grain yield suggest that continued advancement could be made in selecting for increased grain yield under heat stress.

The significant positive SCA effects for grain yield that were observed under managed heat stress, optimal conditions, and across environments showed a significant deviation from what was expected based on the performance of the parents. The results of this study are in line with those of Jodage et al. and Akula et al. [7,13], who identified specific combiners for grain yield under heat stress conditions. The positive significant SCA observed on crosses, DJ265-15× VL1018816, and DJ267-9× CAL1440 implies that the crosses had desirable grain yield alleles for heat stress [34]. The genotypes with high and positive significant SCA estimates from the current study could be used in pedigree starts or doubled haploid in further breeding for heat tolerance in maize in SSA [46].

Information on *per se* performance of the inbred lines is vital for establishing a yield improvement programme in plant breeding. Breeders are interested in developing hybrids from vigorous inbred lines that have good *per se* performance and in cross combinations [50]. This study identified the exotic HSTDLs CAL14138, CAL152, and VL109126 as the best donor lines, as they showed significant GCA and superior *per se* performance under heat stress. The *per se* performance of CAL1440, which was among the best combiners, was not pleasing, as it was slightly above the trial mean as compared to other donors that showed high yield potential. This means that the lines CAL14138 and CAL152 can be effectively used in introgression crosses with local lines, while CAL1440 needs further improvement to enhance its tolerance to heat stress. The exotic HSTDL CAL1440 with average *per se* performance may require crossing with other donor lines, such as CAL14138 and CAL152, which showed good *per ser* performance and positive significant GCA under managed heat stress conditions. The lines with superior *per ser* performance are potential breeding lines that can be used in gene pyramiding schemes with other exotic donor lines. Gene pyramiding through crossing heat-tolerant exotic-by-exotic or exotic-by-local lines can enhance heat tolerance in local material. Additionally, heat-tolerant donor lines can be exposed to gamma irradiation for induction to enhance heat tolerance in sub-tropical breeding material. Good performance exhibited by the exotic HSTDLs, CAL152, and VL109126 across all the test locations shows their wide adaptation; hence the lines can be used in the breeding programme to produce high yielding and adaptable heat-tolerant inbred lines. Parents with high GCA effect have greater adaptability and are less affected by the environment [20]. The exotic HSTDL VL1010762, ZL111056, and CAL14113, which exhibited poor *per se* performance under heat stress, can also be used as heat donors in the introgression of heat tolerance into local material.

Results of this study show that the number of days to 50% anthesis and ASI of the inbred lines shortened under managed heat stress conditions. The shortened flowering period could be due to rapid accumulation of heat units [51], leading to the fast initiation of growth phases [52–54]. The shortened ASI observed in this study contradicts with the findings of Osuman et al., Nyombayire et al., and Rezende et. al. [19,22,55], who reported an increase in ASI in maize under heat stress conditions. The increase in ASI in maize under heat stress conditions might be due to pollen shedding way before silk emergence [38]. Additionally, Zinn et al. [51] reported that susceptible genotypes allocate less assimilates to the developing ears during stress, and this delays silk appearance. Researchers affirmed the role of heat stress in the lengthening of ASI and considered it an indicator of poor tolerance to drought and heat stress [56], but the materials used in this study differ in this trait.

3.5 Conclusion and Recommendations

This study demonstrated the potential of exotic HSTDLs from India in improving the adaptation of maize to heat stress in sub-tropical breeding programmes. The information generated can help to optimize the breeding strategy and is useful for laying a foundation for heat tolerance breeding programmes in SSA. The exotic HSTDLs; CAL14138, CAL152, and CAL1440, that exhibited significant positive GCA effects under heat stress, could be used as important genetic resources for the introgression of heat-tolerant favorable alleles into local maize populations. Single crosses, DJ265-15 × VL1018816, and DJ267-10 × CAL1440 that show high positive and significant SCA effects for grain yield under heat stress conditions can be used for further breeding and contribute to grain yield performance under heat stress conditions. However, backcrossing is recommended to recover the desirable traits of the local female lines.

References

1. Prasanna, B.M.; Cairns, J.E.; Zaidi, P.H.; Beyene, Y.; Makumbi, D.; Gowda, M.; Magorokosho, C.; Zaman-Allah, M.; Olsen, M.; Das, A.; et al. Beat the stress: Breeding for climate resilience in maize for the tropical rainfed environments. *Theor. Appl. Genet.* 2021, *134*, 1729–1752. https://doi.org/10.1007/s00122-021-03773-7.
2. Wahid, A.; Gelani, S.; Ashraf, M.; Foolad, M.R. Heat tolerance in plants: An overview. *Environ. Exp. Bot.* 2007, *61*, 199–223. https://doi.org/10.1016/j.envexpbot.2007.05.011.
3. Smith, K.L. *Ohio Agronomy Guide for Corn Production*; Ohio State University: Columbus, GA, USA, 1996.
4. Badu-Apraku, B.; Fakorede, M.A.B. Improvement of Early and Extra-Early Maize for Combined Tolerance to Drought and Heat Stress in Sub-Saharan Africa. In *Advances in Genetic Enhancement of Early and Extra-Early Maize for Sub-Saharan Africa*; Springer: Cham, Switzerland, 2017; Volume 12, pp. 318–358.
5. Khodarahmpour, Z.; Choukan, R.; Bihamta, M.R.; Majidi-Hervan, E. Determination of the best heat stress tolerance indices in maize (*Zea mays* L.) inbred lines and hybrids under Khuzestan Province conditions. *J. Agric. Sci. Technol.* 2011, *13*, 111–121.
6. Intergovernmental Panel on Climate Change. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth ASSESSMENT Report. 2016. Available online: https://www.ipcc.ch/site/assets/uploads/2018/05/SYR_AR5_FINAL_full_wcover.pdf (accessed on).20 January, 2023.
7. Jodage, K.; Kuchanur, P.H.; Zaidi, P.H.; Patil, A.; Seetharam, K.; Vinayan, M.T.; Arunkumar, B. Genetic analysis of heat stress tolerance and association of traits in tropical maize (*Zea mays* L.). *Environ. Ecol.* 2017, *35*, 2354–2360.
8. Deryng, D.; Conway, D.; Ramankutty, N.; Price, J.; Warren, N. Global crop yield response to extreme heat stress under multiple climate change futures. *Environ. Res. Lett.* 2014, *9*, 034011. https://doi.org/10.1088/1748-9326/9/3/034011.

9. Rani, N.; Nirala, R.B.R.; Pal, A.K.; Ranjan, T. Combining ability for quantitative traits under normal and heat stress conditions in maize (*Zea mays* L.). *Bangladesh J. Bot.* 2021, *50*, 659–669. https://doi.org/10.3329/bjb.v50i3.55846.
10. Cairns, J.E.; Sonder, K.; Zaidi, P.H.; Verhulst, N.; Mahuku, G. Maize Production in a Changing Climate: Impacts, Adaptation, and Mitigation Strategies. *Adv. Agron.* 2012, *114*, 1–58. https://doi.org/10.1016/B978-0-12-394275-3.00006-7.
11. Gazala, P.; Kachanur, P.; Zaidi, P.H.; Amnkumar, B.; Ayyanagouda, P.; Seetharam, K.; Vanayan, M.T. Combining ability and heterosis for heat stress tolerance in maize (*Zea mays* L.). *Bangladesh J. Plant Breed. Genet.* 2018, *21*, 21–28. https://doi.org/10.3329/bjpbg.v21i1.17045.
12. Byerlee, D.; Edmedes, G.O. *Fifty Years of Maize Research in the Cgiar: Diversity, Change and Ultimate Success*; CIMMYT: Mexico City, México, 2021; p. 68.
13. Akula, D.; Patil, A.; Zaidi, P.H.; Kuchanur, P.H.; Vinayan, M.T.; Seetharam, K. Line x testers analysis of tropical maize inbred lines under heat stress for grain yield and secondary traits. *Maydica* 2016, *61*, 3–6.
14. Naveed, M.; Ahsan, M.; Akram, H.M.; Aslam, M.; Ahmed, N. Genetic effects conferring heat tolerance in a cross of tolerant × susceptible maize (*Zea mays* L.) genotypes. *Front. Plant Sci.* 2016, *7*, 729. https://doi.org/10.3389/fpls.2016.00729.
15. Jodage, K.; Kuchanur, P.H.; Zaidi, P.H.; Patil, A.; Seetharam, K.; Vinayan, M.T.; Arunkumar, B. Genetic Analysis of Heat Adaptive Traits in Tropical Maize (*Zea mays* L.). *Int. J. Curr. Microbiol. Appl. Sci.* 2018, *7*, 3237–3246. https://doi.org/10.20546/ijcmas.2018.701.387.
16. Acquaah, G. *Principles of Plant Genetics and Breeding*; Wiley-Blackwell: Baltimore, MD, USA, 2020; p. 63.
17. Umar, U.U.; Ado, S.G.; Aba, D.A.; Bugaje, S.M. Combining Ability, Gene Action and Heterosis in Maize (*Zea mays* L.) under Drought Stress. *Int. J. Agric. Innov. Res.* 2014, *3*, 934–939.
18. Begna, T. Role and economic importance of crop genetic diversity in food security. *Int. J. Agric. Sci. Food Technol.* 2021, *7*, 164–169. https://doi.org/10.17352/2455-815x.000104.
19. Osuman, A.S.; Badu-Apraku, B.; Ifie, B.E.; Nelimor, C.; Tongoona, P. Combining Ability and Heterotic Patterns of Tropical Early-Maturing Maize Inbred Lines under Individual and Combined Heat and Drought Environments. *Plants* 2022, *11*, 1365. https://doi.org/10.3390/plants11101365.
20. Begna, T. Combining ability and heterosis in plant improvement. *Open J. Plant Sci.* 2021, *6*, 108–117. https://doi.org/10.17352/ojps.000043.

21. Chapman, S.C.; Chakraborty, S.; Dreccer, M.F.; Howden, S.M. Plant adaptation to climate change opportunities and priorities in breeding. *Crop Pasture Sci.* 2012, *63*, 251–268. https://doi.org/10.1071/CP11303.
22. Nyombayire, A.; Derera, J.; Sibiya, J.; Ngaboyisonga, C. Heterotic Groups, Gene Action and Heterosis among Maize Inbred Lines Selected for the Major Agro-ecologies of Rwanda. *J. Plant Sci.* 2021, *9*, 245. https://doi.org/10.11648/j.jps.20210905.12.
23. Hallauer, A.R.; Marcelo, J.C.; Miranda, J.B. *Quantitative Genetics in Maize Breeding*, 2nd ed.; Iowa State University Press: Ames, IA, USA, 2010.
24. VSN. *International. GenStat for Windows*, 20th ed.; VSN International: Hemel Hempstead, UK, 2011. Available online: http://www.genstat.co.uk (accessed on).- 10 January 2023
25. Steel, R.G.; Torrie, J.H. *Principles and Procedures of Statistics: A Biometrical Approach*; American Statistical Association Stable: Alexandria, VA, USA, 2014; Volume 76, pp. 753–754. Available online: http://www.jstor.org/stable/2287561 (accessed on).20 December 2022.
26. Barreto, H.J.; Edmeades, G.O. *The Alpha Lattice Design in Plant Breeding and Agronomy: Generation and Analysis*l; CIMMYT: Mexico City, Mexico, 1997. Available online: https://www.semanticscholar.org/paper/Thealpha-lattice-design-in-plant-breeding-and-and-Barreto-Edmeades/65d3efaaaee5396eef88d580c2799c5b448df6d2 (accessed on 19 August 2021).
27. Alvarado, G.; Rodríguez, F.M.; Pacheco, A.; Burgueño, J.; Crossa, J. META-R: A software to analyze data from multi-environment plant breeding trials. *Crop J.* 2020, *8*, 745–756. https://doi.org/10.1016/j.cj.2020.03.010.
28. Rodríguez, F.; Alvarado, G.; Pacheco, A.; Crossa, J.; Burgueno, J. AGD-R (Analysis of Genetic Designs with R for Windows) Version 5.0. *CIMMYT Res. Data Softw. Repos. Netw.* 2015, *12*, 53. Available online: https://hdl.handle.net/11529/10202 (accessed on).20 December 2022.
29. Baker, R.J. Issues in diallel analysis. *Crop Sci.* 1978, *18*, 533–536. https://doi.org/10.2135/cropsci1978.0011183X001800040001x.
30. Falconer, D.S.; Mackay, T.F.C. *Introduction to Quantitative Genetics*; John Wiley and Sons: New York, NY, USA, 1989; p. 128.
31. Dabholkar, A.R. *Elements of Biometrical Genetics*; Concept Publishing Company: New Delhi, India, 1992; p. 166.
32. Cobb, J.N.; Juma, R.U.; Biswas, P.S.; Arbelaez, J.D.; Rutkoski, J.; Altin, G.; Hagen, T.; Quinn, M.; Eng, H.N. Enhancing the rate of genetic gain in public-sector plant breeding programs : Lessons

from the breeder's equation. *Theor. Appl. Genet.* 2019, *132*, 627–645. https://doi.org/10.1007/s00122-019-03317-0.

33. Galluzzi, G.; Seyoum, A.; Halewood, M.; Noriega, I.L.; Welch, E.W. The role of genetic resources in breeding for climate change: The case of public breeding programmes in eighteen developing countries. *Plants* 2020, *9*, 1129. https://doi.org/10.3390/plants9091129.
34. Fasahat, P. Principles and Utilization of Combining Ability in Plant Breeding. Biometrics Biostat. *Int. J.* 2016, *4*, 1–22. https://doi.org/10.15406/bbij.2016.04.00085.
35. Chung, P.Y.; Liao, C.T. Identification of superior parental lines for biparental crossing via genomic prediction. *PLoS ONE* 2020, *15*, e0243159. https://doi.org/10.1371/journal.pone.0243159.
36. Nelimor, C.; Badu-Apraku, B.; Tetteh, A.Y.; Assanvo, S.P.N. Assessment of Genetic Diversity for Drought, Heat and Combined Drought and Heat Stress Tolerance in Early Maturing Maize Landraces. *Plants* 2019, *8*, 518. https://doi.org/10.3390/plants8110518.
37. Nelimor, C.; Badu-Apraku, B.; Tetteh, A.Y.; Oliveira, G.; Assanvo, S.P.N. Assessing the potential of extra-early maturing landraces for improving tolerance to drought, heat, and both combined stresses in maize. *Agronomy* 2020, *10*, 318. https://doi.org/10.3390/agronomy10030318.
38. Nasser, L.M.; Badu-Apraku, B.; Gracen, V.E.; Mafouasson, H.N.A. Combining Ability of Early-Maturing Yellow Maize Inbreds under Combined Drought and Heat Stress and Well-Watered Environments. *Agronomy* 2020, *10*, 1585. https://doi.org/10.3390/agronomy10101585.
39. Derera, J.; Tongoona, P.; Vivek, B.S.; Laing, M.D. Gene action controlling grain yield and secondary traits in southern African maize hybrids under drought and non-drought environments. *Euphytica* 2008, *162*, 411–422. https://doi.org/10.1007/s10681-007-9582-4.
40. Ifie, B.E.; Badu-Apraku, B.; Gracen, V.; Danquah, E.Y. Genetic Analysis of Grain Yield of IITA and CIMMYT Early-Maturing Maize Inbreds under *Striga*-Infested and Low–Soil-Nitrogen Environments. *Crop Sci. Soc. Am.* 2015, *55*, 610–623. https://doi.org/10.2135/cropsci2014.07.0470.
41. Oyekunle, M.; Badu-Apraku, B. Genetic Analysis of Grain Yield and Other Traits of Early-Maturing Maize Inbreds under Drought and Well-Watered Conditions. *Agron. Crop Sci.* 2014, *200*, 92–107. https://doi.org/10.1111/jac.12049.
42. Obeng-Bio, E. *Genetic Analysis of Grain Yield and Other Traits of Early Maturing Provitamin A-Quality Maize Inbred Lines under Drought and Low Soil Nitrogen Conditions*. University of Ghana: Accra, Ghana, 2018.

43. Agaba, R.; Tukamuhabwa, P.; Rubaihayo, P.; Mwanga, R.O.M.; Ssenyonjo, A.; Ndirigwe, J.; Tumwegamire, S.; Gruneberg, W. Heritability, Combining Ability and inheritance of storage root dry matter in Yam Beans. *Afr. Crop Sci.* 2017, *25*, 83–95. http://doi.org/10.4314/acsj.v25i1.6.
44. Smale, M.; Byerlee, D.; Jayne, T. Maize Revolutions in Sub-Saharan Africa. In *An African Green Revolution*; Otsuka, K., Larson, D., Eds.; Springer: Dordrecht, The Netherlands, 2013. https://doi.org/10.1007/978-94-007-5760-8_8.
45. Zaidi, P.H.; Zaman-Allah, M.; Trachsel, S.; Seetharam, K.; Cairns, E.J.; Vanayan, M.T. *Phenotyping for Abiotic Stresstolerance in Maize Heat Stress: A Field Manual*; CIMMYT: Hyderabad, India, 2016.
46. Jha, U.C.; Bohra, A.; Singh, N.P. Heat stress in crop plants: Its nature, impacts and integrated breeding strategies to improve heat tolerance. *Plant Breed.* 2014, *133*, 679–701. https://doi.org/10.1111/pbr.12217.
47. Gissa, D.W.; Zelleke, H.; Labuschagne, M.T.; Hussien, T.; Singh, H. Heterosis and combining ability for grain yield and its components in selected maize inbred lines. *S. Afr. J. Plant Soil* 2007, *24*, 133–137. https://doi.org/10.1080/02571862.2007.10634795.
48. Muluneh, A.N.; Odong, T.L. Combining Ability Analysis and Determination of Controlling Gene Action for Yield and Other Key Traits of Open Pollinated Maize (*Zea Mays* L.) Varieties and their Topcrosses with Inbred Line Testers in Uganda. *Glob. J. Agric. Res.* 2020, *8*, 39–47.
49. Jacobson, A.; Lian, L.; Zhong, S.; Bernardo, R. General Combining Ability Model for Genomewide Selection in a Biparental Cross. *Crop Sci. Soc. Am.* 2014, *54*, 895–905. https://doi.org/10.2135/cropsci2013.11.0774.
50. Ikisa, A. Varietal and hybrid seed production: In agric informatics and services. 2022. Available online: https://www.ikisan.com/ap-maize-varietal-and-hybrid.html (accessed on 28 July 2023).
51. Basavaraj, P.S.; Rane, J. Avenues to realize potential of phenomics to accelerate crop breeding for heat tolerance. *Plant Physiol. Rep.* 2020, *25*, 594–610. https://doi.org/10.1007/s40502-020-00552-2.
52. Zinn, K.E.; Tunc-Ozdemir, M.; Harper, J.F. Temperature stress and plant sexual reproduction: Uncovering the weakest links. *J. Exp. Bot.* 2010, *61*, 1959–1968. https://doi.org/10.1093/jxb/erq053.
53. Aiken, R. Climate change impacts on crop growth in the central high plains. In Proceedings of the 21st Annual Central Plains Irrigation Conference, Colby, KS, USA, 2009; pp. 14–24; Colorado State University Libraries: Fort Collins, CO, USA, 2009.

54. Tao, Z.Q.; Chen, Y.Q.; Chao, L.I.; Zou, J.X.; Peng, Y.A.; Yuan, S.F.; Xia, W.U.; Peng, S.U. The causes and impacts for heat stress in spring maize during grain filling in the North China Plain—A review. *J. Integr. Agric.* 2016, *15*, 2677. https://doi.org/10.1016/S2095-3119(16)61409-0.
55. Manoj, K.; Ghimire, S.K.; Ojha, B.R.; Shrestha, J. Genetic diversity for heat tolerant related traits in maize inbred lines. *Agricultura* 2018, *105*, 23–34. https://doi.org/10.15835/agrisp.v105i1-2.13038.
56. Rezende, W.S.; Beyene, Y.; Mugo, S.; Ndou, E.; Gowda, M.; Sserumaga, J.P.; Asea, G.; Ngolinda, I.; Jumbo, M.; Oikeh, S.O.; et al. Performance and yield stability of maize hybrids in stress-prone environments in eastern Africa. *Crop J.* 2020, *8*, 107–118. https://doi.org/10.1016/j.cj.2019.08.001.

Appendix 0.1 Specific combining ability (SCA) effects of heat tolerant donors and CIMMYT-Zimbabwe elite lines evaluated under heat stress and optimal conditions during 2020 winter season.

		Managed Heat		**Optimal Management**		**Across**	
Entry	**Cross**	**SCA (tha-1)**	**Rank**	**SCA (tha-1)**	**Rank**	**SCA (tha-1)**	**Rank**
171	DJ265-15 x VL1018816	1.05**	1	0.03	84	0.53	18
30	DJ267-9 x CAL1440	0.73*	2	0.7	24	0.74	8
2	DJ267-9 x CAL14138	0.7	3	-0.21	107	0.2	56
123	DJ267-9 x ZL1312	0.67	4	0.93	12	0.86	4
16	DJ265-15 x CAL1412	0.62	5	0.3	53	0.49	23
93	DJ267-6 x VL1010762	0.6	6	-0.23	114	0.17	63
81	DJ265-14 x VL143518	0.6	7	0.39	44	0.52	19
109	DJ267-5 x CAL14113	0.54	8	1	9	0.83	5
102	DJ265-15 x VL1010762	0.54	9	0.63	29	0.62	14
37	DJ265-15 x CAL1440	0.53	10	-0.43	133	0	97
23	DJ194-3 x CAL1412	0.51	11	-0.15	100	0.17	64
66	DJ267-5 x VL109126	0.45	12	-0.48	136	-0.04	103
49	DJ265-13 x ZL132077	0.44	13	-0.26	118	0.08	81
53	DJ194-3 x VL109126	0.44	14	0.16	71	0.31	40
166	DJ194-10 x VL1018816	0.44	15	0.86	14	0.68	10
165	DJ267-5 x VL1018816	0.42	16	0.53	32	0.51	20
63	DJ194-2 x CAL1469	0.42	17	0.05	81	0.22	55
159	DJ265-14 x CAL152	0.42	18	-0.48	137	-0.06	109
112	DJ265-6 x CAL14113	0.42	19	-0.48	135	-0.05	105
122	DJ265-14 x ZL1312	0.41	20	-0.22	111	0.09	79
61	DJ267-9 x C AL1469	0.41	21	-0.7	153	-0.2	125
110	DJ267-8 x CAL14113	0.4	22	0.39	43	0.42	29
18	DJ267-7 x CAL1412	0.38	23	0.04	83	0.23	52
6	DJ267-8 x CAL14138	0.38	24	-0.14	97	0.07	82
11	DJ265-10 x CAL14138	0.36	25	1.34*	4	0.89*	1
41	DJ265-8 x ZL132077	0.35	26	0.23	.62	0.31	41
148	DJ265-15 x ZL111056	0.35	27	-0.32	122	-0.01	100
28	DJ265-15 x CAL1440	0.34	28	0.26	58	0.29	45
170	DJ265-8 x VL1018816	0.34	29	1.09	8	0.77	6
103	DJ267-8 x VL1010762	0.33	30	-0.4	130	-0.04	102
72	DJ265-13 x VL109126	0.32	31	0.15	74	0.24.	51
9	DJ194-2 x CAL14138	0.31	32	0.74	20	0.55	17
17	DJ265-8 x CAL1412	0.31	33	0.14	75	0.25	50
65	DJ265-14 x CAL1469	0.3	34	-0.22	110	0.01	95
83	DJ267-5 x CAL14135	0.3	35	0.24	60	0.3	43

74	DJ265-10 x VL143518	0.3	36	0.29	54	0.32	37
120	DJ194-2 x CAL14113	0.29	37	0.46	40	0.4	31
107	DJ267-7 x VL143518	0.29	38	0.57	30	0.46	25
124	DJ265-15 x ZL1312	0.29	39	-0.25	115	0.01	93
14	DJ265-15 x CAL14138	0.28	40	0.5	34	0.38	33
7	DJ194-10 x CAL14138	0.28	41	0.47	38	0.38	34
132	DJ265-15 x CAL14113	0.26	42	-0.19	106	0.03	88
172	DJ267-8 x VL1018816	0.26	43	0.12	78	0.2	59
76	DJ267-9 x VL143518	0.25	44	0.76	19	0.55	16
43	DJ267-9 x ZL132077	0.25	45	0.12	77	0.19	60
126	DJ267-8 x ZL1312	0.25	46	-0.18	104	0.04	86
108	DJ265-8 x VL143518	0.24	47	0.97	10	0.67	12
137	DJ265-13 x ZL111056	0.24	48	-0.95	163	-0.41	146
135	DJ267-6 x ZL111056	0.24	49	0.25	59	0.25	49
152	DJ267-5 x CAL152	0.23	50	0.18	66	0.2	57
168	DJ267-7 x VL1018816	0.23	51	0.02	86	0.14	70
96	DJ267-5 x VL1010762	0.22	52	0.3	52	0.29	47
154	DJ265-6 x CAL152	0.21	53	0.17	68	0.18	62
118	DJ267-9 x CAL14113	0.2	54	-0.17	102	0.02	92
73	DJ265-6 x VL109126	0.2	55	0.85	15	0.56	15
47	DJ194-10 x ZL132077	0.19	56	0.71	23	0.5	21
92	DJ265-8 x CAL14135	0.19	57	0.03	85	0.13	71
79	DJ267-5 x VL143518	0.18	58	-1.03	166	-0.47	149
97	DJ194-10 x VL1010762	0.18	59	0.65	27	0.46	28
129	DJ265-8 x ZL1312	0.18	60	0.18	67	0.2	58
70	DJ265-10 x VL109126	0.18	61	1.42*	2	0.88*	2
156	DJ265-10 x CAL152	0.18	62	-0.34	127	-0.12	114
88	DJ194-10 x CAL14135	0.18	63	1.12	7	0.73	9
69	DJ267-9 x VL109126	0.18	64	1.19	6	0.75	7
24	DJ265-14 x CAL1412	0.17	65	0.04	82	0.13	72
104	DJ194-3 x VL1010762	0.15	66	0.15	72	0.17	66
82	DJ267-7 x CAL14135	0.15	67	-0.15	98	0.01	94
38	DJ194-2 x CAL1440	0.13	68	0.8	17	0.49	24
64	DJ265-13 x CAL1469	0.12	69	1.49*	1	0.86	3
157	DJ265-15 x CAL152	0.12	70	0.5	35	0.31	39
136	DJ265-6 x ZL111056	0.12	71	0.42	41	0.29	46
36	DJ194-3 x CAL1440	0.11	72	-0.21	108	-0.09	112
45	DJ265-14 x ZL132077	0.11	73	-0.23	113	-0.06	108
160	DJ194-3 x CAL152	0.1	74	-0.36	129	-0.18	120
	DJ194-3 x VL1018816	0.09	75	-0.04	92	0.02	90

140	DJ194-10 x ZL111056	0.09	76	0.11	79	0.1	78
101	DJ265-10 x VL1010762	0.08	77	-0.62	150	-0.29	132
26	DJ265-6 x CAL1440	0.07	78	-1.2	171	-0.65	160
1	DJ265-6 x CAL14138	0.06	79	0.42	42	0.23	53
67	DJ265-15 x VL109126	0.06	80	-0.15	99	-0.05	104
4	DJ265-8 x CAL14138	0.06	81	-0.51	140	-0.28	131
161	DJ265-8 x CAL152	0.06	82	0.88	13	0.5	22
173	DJ265-15 x VL1018816	0.06	83	-0.18	105	-0.08	111
144	DJ265-8 x ZL111056	0.05	84	0.16	69	0.12	73
85	DJ265-6 x CAL14135	0.05	85	-0.33	124	-0.14	116
68	DJ194-2 x VL109126	0.04	86	0.3	51	0.19	61
113	DJ267-6 x CAL14113	0.04	87	0.77	18	0.46	26
54	DJ265-8 x VL109126	0.03	88	-0.34	125	-0.18	121
130	DJ265-10 x ZL1312	0.02	89	-0.01	87	0.02	91
145	DJ265-15 x ZL111056	0.01	90	0.27	57	0.15	68
46	DJ265-10 x ZL132077	0.01	91	0.69	25	0.4	32
87	DJ265-10 x CAL14135	0	92	-0.29	119	-0.13	115
42	DJ267-5 x ZL132077	0	93	0.27	56	0.17	65
139	DJ267-5 x ZL111056	0	94	0.06	80	0.04	87
22	DJ194-2 x CAL1412	0	95	0.51	33	0.3	44
121	DJ265-8 x CAL14113	-0.01	96	0.56	31	0.31	38
33	DJ265-10 x CAL1440	-0.01	97	0.15	73	0.06	84
99	DJ267-7 x VL1010762	-0.01	98	0.72	21	0.42	30
40	DJ267-5 x ZL1312	-0.02	99	-0.1	95	-0.06	106
5	DJ267-6 x CAL14138	-0.04	100	0.35	48	0.15	69
58	DJ267-7 x CAL1469	-0.05	101	1.22	5	0.64	13
57	DJ194-10 x CAL1469	-0.05	102	0.49	36	0.23	54
75	DJ265-15 x VL143518	-0.05	103	0.64	28	0.34	35
12	DJ265-15 x CAL14138	-0.05	104	-0.52	142	-0.33	139
89	DJ267-9 x CAL14135	-0.06	105	0.48	37	0.26	48
39	DJ267-6 x ZL132077	-0.06	106	-0.26	116	-0.16	119
134	DJ194-2 x VL143518	-0.06	107	-0.31	121	-0.2	124
116	DJ265-15 x CAL14113	-0.06	108	-0.31	120	-0.19	123
82	DJ265-13 x CAL14135	-0.07	109	-0.7	152	-0.39	144
158	DJ194-10 x CAL152	-0.07	110	0.23	61	0.08	80
142	DJ267-7 x ZL111056	-0.07	111	-0.87	161	-0.5	150
20	DJ267-9 x CAL1412	-0.07	112	-0.23	112	-0.15	117
60	DJ265-10 x CAL1469	-0.08	113	-0.08	94	-0.09	113
91	DJ265-15 x CAL14135	-0.09	114	-0.52	141	-0.31	134
151	DJ265-15 x CAL152	-0.1	115	0.71	22	0.33	36

35	DJ194-10 x CAL1440	-0.1	116	0.29	55	0.1	77
34	DJ267-8 x CAL1440	-0.1	117	1.35*	3	0.68	11
138	DJ265-10 x ZL111056	-0.11	118	0.22	63	0.06	83
147	DJ194-3 x ZL111056	-0.12	119	0.93	11	0.46	27
8	DJ267-5 x CAL14138	-0.12	120	-0.26	117	-0.21	126
25	DJ267-6 x CAL1440	-0.13	121	-0.34	126	-0.27	129
150	DJ267-6 x CAL152	-0.14	122	-0.35	128	-0.28	130
153	DJ267-9 x CAL152	-0.15	123	0.13	76	-0.02	101
141	DJ267-9 x ZL111056	-0.15	124	0.32	49	0.11	74
133	DJ267-6 x VL143518	-0.16	125	-0.03	89	-0.07	110
59	DJ267-6 x CAL1469	-0.17	126	-0.43	131	-0.33	137
162	DJ267-6 x VL1018816	-0.17	127	-0.6	149	-0.42	147
115	DJ265-10 x CAL14113	-0.2	128	-0.57	147	-0.39	143
131	DJ267-7 x ZL1312	-0.2	129	-0.53	144	-0.39	142
174	DJ265-13 x VL1018816	-0.21	130	0.2	64	0	96
90	DJ194-3 x CAL14135	-0.22	131	-0.43	132	-0.32	136
143	DJ265-14 x ZL111056	-0.22	132	-0.5	138	-0.38	140
106	DJ265-14 x VL1010762	-0.22	133	0.38	45	0.11	76
32	DJ265-14 x CAL1440	-0.22	134	-0.12	96	-0.18	122
13	DJ194-3 x CAL14138	-0.24	135	-0.83	160	-0.61	158
105	DJ265-15x VL1010762	-0.26	136	0.2	65	-0.01	99
94	DJ265-6 x VL1010762	-0.26	137	-0.69	151	-0.5	151
149	DJ194-2 x ZL111056	-0.27	138	-0.18	103	-0.23	128
31	DJ267-7 x CAL1440	-0.29	139	0.46	39	0.11	75
146	DJ267-8 x ZL111056	-0.29	140	-0.04	91	-0.16	118
71	DJ267-6 x VL109126	-0.29	141	-0.88	162	-0.62	159
167	DJ267-9 x VL1018816	-0.3	142	0.35	47	0.05	85
44	DJ265-6 x ZL132077	-0.31	143	-0.47	134	-0.4	145
169	DJ265-14 x VL1018816	-0.31	144	0.16	70	-0.06	107
27	DJ265-13 x CAL1440	-0.32	145	0.36	46	0.02	89
164	DJ265-10 x VL1018816	-0.32	146	-0.79	158	-0.6	156
128	DJ265-8 x ZL1312	-0.33	147	0.8	16	0.3	42
29	DJ267-5 x CAL1440	-0.34	148	-1	165	-0.73	163
19	DJ267-6 x CAL1412	-0.34	149	-0.71	154	-0.55	153
119	DJ265-14 x CAL14113	-0.35	150	-1.15	170	-0.8	165
62	DJ265-6 x CAL1469	-0.36	151	-0.07	93	-0.22	127
10	DJ265-14 x CAL14138	-0.38	152	0.68	26	0.16	67
111	DJ267-7 x CAL14113	-0.38	153	-0.75	156	-0.59	155
155	DJ267-7 x CAL152	-0.39	154	-0.22	109	-0.32	135
48	DJ194-3 x ZL132077	-0.39	155	-0.76	157	-0.6	157

114	DJ194-3 x CAL14113	-0.4	156	0.3	50	-0.01	98
80	DJ194-3 x VL143518	-0.41	157	-0.57	146	-0.51	152
51	DJ267-7 x VL109126	-0.43	158	-1.08	167	-0.81	166
98	DJ267-9 x VL1010762	-0.45	159	-0.16	101	-0.29	133
78	DJ194-10 x VL143518	-0.49	160	-0.81	159	-0.68	161
95	DJ265-13x VL1010762	-0.5	161	-1.32*	173	-0.96*	169
100	DJ265-8 x VL1010762	-0.58	162	-0.32	123	-0.46.	148
86	DJ267-6 x CAL14135	-0.62	163	-0.52	143	-0.57	154
175	DJ194-2 x VL1018816	-0.64	164	-1.08	168	-0.91*	167
55	DJ267-5 x CAL1469	-0.66	165	-1.25	172	-1.03*	171
3	DJ265-13 x CAL14138	-0.69	166	-0.03	90	-0.39	141
15	DJ267-5 x CAL1412	-0.7	167	-0.02	88	-0.33	138
50	DJ194-2 x ZL132077	-0.79*	168	-0.58	148	-0.7	162
117	DJ194-10 x CAL14113	-0.94**	169	-0.51	139	-0.74	164
77	DJ265-15 x VL143518	-0.95*	170	-1.33*	174	-1.21**	173
163	DJ265-6 x VL1018816	-1.02**	171	-0.74	155	-0.92*	168
52	DJ265-14 x VL109126	-1.04**	172	-1.12	169	-1.14**	172
21	DJ265-6 x CAL1412	-1.25***	173	-0.55	145	-0.98*	170
127	DJ265-6 x ZL1312	-1.49***	174	-0.98	164	-1.34**	174

CHAPTER 4

Genetic diversity, relatedness and purity of CIMMYT-India exotic heat tolerant maize donor lines and CIMMYT-Zimbabwe elite lines using SNP markers

Abstract

Genotyping of breeding materials for genetic diversity, relationship, and purity assessment should be a part of every efficient crop improvement programme for better selection of parents to use in line and hybrid development. Assessment of genetic purity is a crucial quality control measure. The current study assessed genetic diversity, relatedness, and purity of 35 inbred lines (that is, 14 heat tolerant donor lines from CIMMYT-India and 21 elite lines from CIMMYT-Zimbabwe) using 94 single-nucleotide polymorphism (SNP) markers following the Kompetitive allele-specific polymerase chain reaction (KASP™) genotyping protocol. Majority (85.78%) of the inbred lines assessed were genetically pure with more than 95% homozygosity. About 54.28% of the inbred lines had genetic purity of 100%. Out of the 35 inbred lines that were evaluated, 5 (CZL15033 (85.7%), CZL15038 (83%), DJ611-7 (83.7%), CAL440 (93.9%) and CML576 (87.7%) failed the purity test. The identity-by-state (IBS) genetic distance matrix revealed pairwise genetic distance among the inbred lines ranging from 0.04 to 0.64, demonstrating a considerable degree of variation between the inbred lines. The widest genetic distance of 0.64 was observed between inbred line pairs: CZL1112c and CZL16018; CAL14135 and ZL132077; and CZL15153 and CZL16018. The shortest genetic distance was observed between inbred lines: CAL152 and CAL14138 (0.04); CAL14138 and VL109126 (0.04); and ZL132077 and DJ611-1 (0.05). The neighbor-joining algorithm implemented in molecular evolutionary genetics analysis (MEGA) grouped inbred lines into three different main clusters, which can potentially serve as the basis for heat stress tolerance breeding. A local inbred line DJ611-4 clustered together with CAL1440 while CML566 clustered together with CAL14138, CAL152 and VL109126 exotic lines. These results imply that within the CIMMYT-Zimbabwe program, there are valuable inbred lines that can be used in heat stress tolerance breeding in SSA.

Key words: Genetic diversity, genetic purity, single nucleotide polymorphism, genetic distance, cluster analysis

4.1 Introduction

Globally, maize is the most important and widely produced cereal crop (Huma et al. 2019), yet an array of biotic and abiotic stressors negatively affect its production in many developing countries. The main concern currently challenging maize production is the global climate change (Cairns et al. 2012). Heat stress is one of the climate change-related factors that has significantly reduced yields in SSA (Deryng et al. 2014; Jodage et al. 2017; Archana et al. 2018). Depending on the frequency, length, and intensity of the heat stress, grain yield loss have been observed to reach 70% and even 100% (Khodarahmpour et al. 2011; Jodage et al. 2018). Genetic resistance has been recognized as the most viable and sustainable approach to improve maize productivity under heat stress conditions. CIMMYT-Zimbabwe has a collection of elite maize inbred lines that have been extensively used in the development of high-yielding maize hybrids. Combining the heat tolerant donor lines from Indian with the elite lines from Zimbabwean could produce heat-tolerant, high-performing maize hybrids. However, to maximize the effectiveness of such breeding efforts, it is important to first understand the genetic relationships and diversity within and between these lines.

The effectiveness of a breeding programme is greatly dependent on genetic diversity (variability of genetic information present within and among populations) of the breeding material, which forms the foundation for crop improvement (Ali et al. 2008; Nyombayire et al. 2016). Identification of appropriate parental lines to produce superior hybrids and progenies with maximum genetic variability has been made easier with the use of information from genetic diversity and relatedness analysis amongst maize inbred lines (Semagn et al. 2012a; Semagn et al. 2012b; Adekemi et al. 2020). Moreover, genetic diversity data is useful in designing a breeding strategy and expanding the genetic base of maize germplasm across various breeding programmes (Ajala et al. 2019). As the primary quality control genotyping criterion that bestows excellent quality on the seed, genetic purity (the degree of genetic uniformity or homogeneity within a line, or hybrid) assessment is an essential component in a breeding programme (Semagn et al. 2012a). Furthermore, because many countries have strict intellectual property laws governing plant breeding and variety registrations, genetic purity evaluations becomes essential for breeding programmes (Semagn et al. 2012a).

An inbred line's genetic purity can be confirmed through grow out test, the use biochemical markers, or the use of molecular markers (Fernandez et al. 2023). Phenotypic and genomic approaches have been used to assess genetic diversity and relatedness of germplasm. Morphological markers have been used for phenotypic evaluations (Semagn et al. 2012a), whereas molecular markers have been used for genomic level research (Semagn et al. 2012a; Semagn et al. 2012b; Nadeem et al. 2018; Adekemi et al. 2020). Morphological markers have been frequently employed to measure genetic diversity, but their polymorphism is limited, the data are prone to environmental bias, and the procedure is time-consuming and expensive (Mafakheri et al. 2017; Ajala et al. 2019). Molecular markers, on the other hand, are the best to draw conclusions about genetic diversity between genotypes because they are highly polymorphic, stable, reproducible, and readily available in the genome. They can be measured throughout all developmental stages, have known positions in the genome, can handle a large number of entries, are not affected by the environment (Govindaraj et al. 2015; Boakyewaa et al. 2019; Silva et al. 2020) and they can save time and money due to their increased efficiency (Ertiro et al. 2015; Mushtaq et al. 2016). The molecular marker method detects variation of the genotypes directly at the deoxyribonucleic acid (DNA) level (Gowda et al. 2017).

Using various genotyping platforms and marker density, molecular marker studies have been carried out to explore the genetic diversity, relationships, population structure, and heterotic grouping of maize inbred lines (Semagn et al. 2012b; Dao et al. 2014; Gowda et al. 2017; Boakyewaa et al. 2019). Polymorphism can be detected using different molecular markers, including amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLPs), simple sequence repeats (SSRs), and single nucleotide polymorphism (SNP), , (Dao et al. 2014). Single nucleotide polymorphism markers are more often used for diversity study than other markers due to their stability, wide distribution, and abundance in the genome (Nelimor et al. 2019; Badu-Apraku et al. 2021). The use of SNP markers has been stressed by contemporary advances in molecular technology because, in contrast to other markers, they are codominant, locus-specific, cost-effective per data point, and have sufficient genomic abundance (Prasanna, 2012; Fernandez et al. 2023).

Although several studies have been conducted to assess the genetic diversity in maize germplasm using SNP markers, genetic diversity analysis of heat tolerant donor lines from exotic gene pools and elite inbred lines from CIMMYT-Zimbabwe has not been conducted, yet this information is important to facilitate effective utilization of the germplasm to design heat stress tolerance breeding programme. The current study was conducted to: (i) assess the magnitude of genetic diversity, genetic relatedness and genetic purity of 14 exotic heat tolerant maize donor lines sourced from CIMMYT- India and 21 elite lines from CIMMYT-Zimbabwe using SNP markers; and (ii) identify elite inbred lines adapted to mid-altitude climatic conditions that can potentially confer heat tolerance in maize breeding programmes in SSA. The study was based on the hypothesis that, among the locally adapted elite lines, germplasm closely related to the exotic heat tolerant donor lines will be identified and used to improve genetic gains of maize under heat stress in SSA.

4.2 Materials and Methods

4.2.1 Plant genetic material, leaf sampling, DNA extraction and genotyping

A total of 35 maize inbred lines, comprising of 14 exotic heat tolerant donor lines sourced from CIMMYT-India and 21 elite inbred lines from CIMMYT-Zimbabwe (Table 4.1) were used in the study. Seeds of the 35 inbred lines were planted in a nursery at CIMMYT-Muzarabani site (Altitude = 343 meters above sea level (masl), GIS = -16^o 39’S, 31^o 01’E, Soil type = red clay soils, average minimum winter temperature = 14^{o}C, average maximum winter temperature = 28^{o}C) in Zimbabwe, during 2019 winter season, in one-row plots of 4 m long. Three to five leaf sample discs were taken for DNA extraction from randomly chosen plants in each of the 35 plots three weeks after crop emergence.

Samples of leaves from the same entry were put in well plates, each plate representing a separate line. A perforated trip cap was used to seal each well, and the plastic lid was placed on top of the desiccant sachet immediately on top of the well that had been sealed with a strip cap. The storage rack was fastened with an elastic band and placed in a sealable plastic bag. The sealed plastic bag was placed into the plant kit box and the samples were dispatched to LGC Genomics Laboratory, in the United Kingdom for genotyping. DNA extraction, amplification and visualization were done following the LGC protocol (www.lgcgroup.com).

Prior to genotyping, DNA was isolated from leaf disc samples and its quality and quantity were assessed. A total of 94 single nucleotide polymorphism (SNP) markers (Table 4.2) were used for genotyping following the Kompetitive Allele Specific Polymerase Chain Reaction (KASP^{TM}) protocol used by LGC Genomics (www.lgcgroup.com) (Semagn et al. 2012b). The markers used are a subgroup of the 100 SNP markers recommended by CIMMYT for routine quality control genotyping in maize and they cover 10 pairs of the maize chromosomes (Gowda et al. 2017).

Table 4.1 Genetic material used for genotyping

No	Line Name	Heterotic group	Grain color	Germplasm Source
1	CZL16018	B	White	CIMMYT-Zimbabwe
2	DJ647-24	A	White	CIMMYT-Zimbabwe
3	DJ647-25	A	White	CIMMYT-Zimbabwe
4	CZL15153	A	White	CIMMYT-Zimbabwe
5	CZL15202	A	White	CIMMYT-Zimbabwe
6	CZL1112c	A	White	CIMMYT-Zimbabwe
7	CZL15033	B	White	CIMMYT-Zimbabwe
8	DJ611-1	A	White	CIMMYT-Zimbabwe
9	CZL15025	B	White	CIMMYT-Zimbabwe
10	DJ475-7	A	White	CIMMYT-Zimbabwe
11	CZL15038	A	White	CIMMYT-Zimbabwe
12	DJ611-4	A	White	CIMMYT-Zimbabwe
13	CZL20002	B	White	CIMMYT-Zimbabwe
14	CZL20003	B	White	CIMMYT-Zimbabwe
15	DJ611-7	B	White	CIMMYT-Zimbabwe
16	CAL14113	B	Yellow	CIMMYT-India
17	CAL1412	A	Yellow	CIMMYT-India
18	CAL14135	B	Yellow	CIMMYT-India
19	CAL14138	A	Yellow	CIMMYT-India
20	CAL1440	A	Yellow	CIMMYT-India
21	CAL1469	A	Yellow	CIMMYT-India
22	CAL152	A	Yellow	CIMMYT-India
23	VL1010762	A	Yellow	CIMMYT-India
24	VL1018816	B	Yellow	CIMMYT-India
25	VL109126	A	Yellow	CIMMYT-India
26	VL143518	B	Yellow	CIMMYT-India
27	ZL111056	A	Yellow	CIMMYT-India
28	ZL1312	B	Yellow	CIMMYT-India
29	ZL132077	A	Yellow	CIMMYT-India
31	CML591	B	White	CIMMYT-Zimbabwe
32	CML566	A	White	CIMMYT-Zimbabwe
33	CML576	A	White	CIMMYT-Zimbabwe
34	CML545	A	White	CIMMYT-Zimbabwe
35	DJ647-22	B	White	CIMMYT-Zimbabwe

Table 4.2 List of 94 SNP markers used to genotype 35 maize inbred lines

No	Marker	No	Marker
1	PZA00175_2	48	PZA02187_1
2	BDIBC025	49	PZA03536_1
3	PZA00495_5	50	PHM4134_8
4	PZA03409_1	51	PHM4080_15
5	PZA01427_1	52	PHM2350_17
6	PZA02462_1	53	PHM2770_19
7	PZA00214_1	54	PHM5572_19
8	PZA01462_1	55	PHM12706_14
9	PZA00084_2	56	PZA01607_1
10	PZA01533_2	57	PZA00793_2
11	PZA03645_1	58	PHM3922_32
12	PZA00770_1	59	PZA01933_3
13	PZA01062_1	60	ae1_7
14	PZB01899_1	61	PZA02378_7
15	PHM5181_10	62	PHM2343_25
16	PZA01791_2	63	PZA02325_4
17	PZA01477_3	64	PZA03120_1
18	PZA02269_3	65	PHM11114_7
19	PZA00527_10	66	PHM15964_16
20	PZA01447_1	67	PHM1968_22
21	PZA02742_1	68	PHM10621_29
22	PZA00399_11	69	PZB01062_3
23	PZA02174_2	70	PHM6111_5
24	PZA01715_2	71	PHM3668_12
25	lac1_3	72	PHM3626_3
26	PHM229_15	73	PZD00022_5
27	PHM4066_11	74	PHM13440_13
28	PZA03211_6	75	PHM17210_5
29	PZA02164_16	76	PZA00667_2
30	PZA00352_23	77	PHM5502_31
31	PHM3457_6	78	PZA02779_1
32	PZA00664_3	79	PZA00726_10
33	PZB01403_1	80	PZA03322_5
34	PZA02436_1	81	PHM662_27
35	PZA00413_20	82	PHM4165_14
36	PHM1752_36	83	PZA00440_1
37	PZA00814_1	84	PZA00355_2
38	PHM5805_19	85	PZA00498_5
39	PZB01658_1	86	PZA02358_1
40	PHM7916_4	87	PZA00218_1
41	PHM3466_69	88	PZA00981_3
42	PHM4752_14	89	PZA00643_13
43	PZB01109_1	90	PZA02480_1
44	PHM3078_12	91	PZA03182_5
45	PZA02090_1	92	PZE-101093951
46	PHM2749_10	93	PZE0186065237
47	PZA01919_2	94	PZE0186365075

4.2.2 Data analysis

A total of 100 SNPs were used in the current research based on earlier studies on maize at CIMMYT. However, 6 SNPs were excluded from the analysis because they were not polymorphic with the inbred lines used. The genetic distance was calculated between the pairs of inbred lines using the identity-by-state (IBS) genetic distance matrix method implemented in the Trait Analysis by Association Evolution and Linkage (TASSEL) software v5.2.24 (Bradbury et al. 2007). Cluster analysis was performed on the genetic distance matrix data using the neighbor-joining algorithm implemented in the molecular evolutionary genetics analysis (MEGA) software v7.035 (Tamura et al. 2013). Percentage residual heterozygosity was used to calculate the genetic purity of the inbred lines, as described by Semagn et al. (2012a) as follows:

$$\text{Genetic purity (GP)\%} = 1 - \frac{\text{NH}}{\text{TSNP} - \text{MNSP}} \text{ X } 100$$

where: NH = number of heterozygous loci (different band nucleotide) of SNP, TSNP = total number of single nucleotide polymorphism in the sample, and MSNP = number of missed single nucleotide polymorphism in the sample

4.3 Results

4.3.1 Genetic distance and relationship among the exotic and the local elite lines

Based on the 94 polymorphic SNPs used, a genetic distance matrix was generated among all the pairs of inbred lines from most closely related to most distant. Pairwise comparisons of genetic distance among the inbred lines revealed that majority (99%) of the lines had genetic distance values above 0.30. About 87% of the inbred lines had genetic distance values between 0.40 and 0.64 (Table 4.3). Only three pairs which is about 0.005% of inbred lines used, showed genetic distance estimates less than 0.05 with two of these pairs originating from CIMMYT-India and one pair with lines from both sources.

The widest genetic distance between the inbred lines was 0.64 and this was observed between inbred lines CAL1435 and ZL132077, CZL112c and CZL16018, and CZL15153 and CZL16018. Among the pairs with highest genetic distance, two pairs consisted of lines from CIMMYT-Zimbabwe while the other pair consisted of lines from CIMMYT-India. It is interesting to note that a good number of

CIMMYT-Zimbabwe lines have wide genetic distance and are in different heterotic groups. The shortest genetic distance was observed between inbred lines CAL152 and CAL14138 (0.04), CAL14138 and VL109126 (0.04), and ZL132077 and DJ611-1 (0.05).

4.4.2 Elite lines adapted to mid-altitude climatic conditions that can potentially confer heat tolerance in maize breeding programmes.

Cluster analysis resolved inbred lines into three distinct clusters (Figure 4. 1). The first cluster (blue) was the largest with 24 (70.6%) inbred lines from both CIMMYT-Zimbabwe and CIMMYT-India sources. The cluster was further partitioned into 2 sub-clusters showing distinct grouping within individual sub-groups with inbred lines from both sources. It is interesting to note that lines from both sources were clustered together and they fall in the same heterotic group (i.e., VL143518, CZL15033, CAL14135 and CZL15025 heterotic group B). In heterotic group A, inbred lines which clustered together were CZL15202, DJ647-24, CAL1469, VL1010762, CAL1440, and CAL1412 and DJ611-4. It is also encouraging to note that DJ611-4 a local line clustered together with exotic line CAL1440 which showed significant and positive general combining ability under heat stress in the previous study (Mukaro et al. 2023)

The second cluster (black) had two sub-clusters comprising of 5 (14.7%) inbred lines from CIMMYT-Zimbabwe that are closely related in their pedigree information and heterotic grouping. The inbred lines in this cluster are all in heterotic group A and these includes CML545, CML576, CZL15038, CZL1112c and CZL15153 (Figure 4.1).

The third cluster (Red) consisted of 5 (14.7%) inbred lines in which four lines were from CIMMYT-India and one from CIMMYT-Zimbabwe. The inbred lines in cluster 3 were CML566, ZL111056, CAL152, VL109126 and CAL14138. It is interesting to note that all lines in this cluster are in heterotic group A. Additionally, CAL566 clustered together with CAL152 and CAL14138, heat donor lines that showed good general combining ability and good *per ser* performance under heat stress in the study conducted by Mukaro et al. (2023).

Table 0.3 Identity-by-State genetic distance estimation between 35 inbred lines using 94 SNP markers

Entry	CZL16018	DJ647-24	DJ647-25	CZL15153	CZL15202	CZL1112c	CZL15033	DJ611-1	CZL15025	DJ475-7	CZL15038	DJ611-4	CZL20002	CZL20003	DJ611-7	CAL14138	CAL1412	CAL1440	ZL132077	VL109126	CAL1469	VL1018816	VL143518	CAL14135	VL1010762	CAL14113	ZL1312	ZL111056	CAL152	CML591	CML566	CML576	CML545
DJ647-24	0.6																																
DJ647-25	0.5	0.5																															
CZL15153	0.6	0.5	0.4																														
CZL15202	0.6	0.4	0.5	0.4																													
CZL1112c	0.6	0.4	0.3	0.3	0.5																												
CZL15033	0.4	0.5	0.5	0.4	0.5	0.4																											
DJ611-1	0.4	0.6	0.4	0.5	0.6	0.5	0.5																										
CZL15025	0.5	0.5	0.3	0.4	0.5	0.3	0.4	0.5																									
DJ475-7	0.5	0.5	0.4	0.4	0.4	0.5	0.4	0.6	0.3																								
CZL15038	0.6	0.4	0.5	0.4	0.5	0.4	0.5	0.6	0.4	0.5																							
DJ611-4	0.4	0.5	0.4	0.6	0.5	0.5	0.5	0.5	0.4	0.6	0.5																						
CZL20002	0.4	0.5	0.5	0.5	0.4	0.5	0.5	0.4	0.5	0.5	0.6	0.5																					
CZL20003	0.1	0.6	0.5	0.5	0.6	0.6	0.4	0.4	0.4	0.5	0.5	0.4	0.4																				
DJ611-7	0.3	0.6	0.4	0.5	0.6	0.5	0.4	0.3	0.4	0.5	0.5	0.5	0.5	0.3																			
CAL14138	0.5	0.5	0.5	0.4	0.4	0.5	0.5	0.6	0.4	0.5	0.5	0.5	0.5	0.4	0.4																		
CAL1412	0.5	0.4	0.5	0.5	0.5	0.4	0.6	0.5	0.4	0.6	0.4	0.3	0.4	0.5	0.6	0.4																	
CAL1440	0.5	0.5	0.4	0.5	0.6	0.4	0.5	0.5	0.4	0.5	0.5	0.4	0.4	0.4	0.5	0.5	0.3																
ZL132077	0.4	0.5	0.4	0.5	0.6	0.5	0.5	0.1	0.5	0.6	0.5	0.4	0.4	0.4	0.3	0.5	0.4	0.5															
VL109126	0.4	0.5	0.5	0.4	0.4	0.5	0.5	0.5	0.4	0.5	0.4	0.6	0.6	0.4	0.4	0.0	0.4	0.5	0.5														
CAL1469	0.5	0.5	0.5	0.5	0.5	0.5	0.4	0.5	0.5	0.4	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.4	0.5	0.4													
VL1018816	0.4	0.4	0.5	0.5	0.5	0.5	0.4	0.6	0.4	0.6	0.5	0.4	0.6	0.6	0.6	0.5	0.5	0.4	0.6	0.5	0.4												
VL143518	0.5	0.5	0.5	0.5	0.4	0.5	0.4	0.5	0.4	0.5	0.5	0.4	0.5	0.6	0.5	0.5	0.5	0.6	0.4	0.5	0.5	0.5											
CAL14135	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.6	0.5	0.4	0.5	0.5	0.5	0.5	0.5	0.5	0.4	0.6	0.6	0.5	0.5	0.6	0.5										

Entry	**CZL16018**	**DJ647-24**	**DJ647-25**	**CZL15153**	**CZL15202**	**CZL1112c**	**CZL15033**	**DJ611-1**	**CZL15025**	**DJ475-7**	**CZL15038**	**DJ611-4**	**CZL20002**	**CZL20003**	**DJ611-7**	**CAL14138**	**CAL1412**	**CAL1440**	**ZL132077**	**VL109126**	**CAL1469**	**VL1018816**	**VL143518**	**CAL14135**	**VL1010762**	**CAL14113**	**ZL1312**	**ZL111056**	**CAL152**	**CML591**	**CML566**	**CML576**	**CML545**
VL1010762	0.4	0.6	0.5	0.5	0.5	0.4	0.5	0.4	0.4	0.5	0.4	0.4	0.4	0.4	0.5	0.5	0.4	0.2	0.4	0.4	0.5	0.5	0.6	0.5									
CAL14113	0.5	0.5	0.5	0.4	0.6	0.5	0.5	0.5	0.5	0.6	0.4	0.4	0.6	0.5	0.5	0.4	0.5	0.4	0.5	0.3	0.4	0.4	0.5	0.5	0.4								
ZL1312	0.4	0.5	0.4	0.5	0.5	0.5	0.5	0.4	0.5	0.5	0.5	0.4	0.5	0.4	0.4	0.4	0.4	0.4	0.3	0.4	0.4	0.5	0.6	0.5	0.3	0.4							
ZL111056	0.5	0.4	0.5	0.5	0.4	0.5	0.5	0.4	0.6	0.6	0.5	0.5	0.5	0.5	0.4	0.3	0.5	0.4	0.4	0.3	0.5	0.5	0.5	0.5	0.5	0.4	0.4						
CAL152	0.5	0.5	0.4	0.4	0.5	0.5	0.5	0.5	0.3	0.5	0.5	0.5	0.5	0.4	0.4	0.0	0.4	0.5	0.5	0.1	0.5	0.5	0.5	0.5	0.4	0.4	0.4	0.3					
CML591	0.5	0.4	0.5	0.5	0.4	0.6	0.5	0.6	0.6	0.4	0.5	0.4	0.5	0.5	0.5	0.5	0.4	0.4	0.5	0.5	0.4	0.3	0.5	0.4	0.5	0.5	0.5	0.5	0.5				
CML566	0.5	0.5	0.4	0.5	0.5	0.4	0.4	0.4	0.4	0.6	0.4	0.4	0.6	0.5	0.5	0.4	0.4	0.5	0.4	0.4	0.6	0.4	0.4	0.5	0.5	0.4	0.4	0.4	0.3	0.6			
CML576	0.5	0.5	0.4	0.4	0.5	0.3	0.4	0.4	0.4	0.5	0.4	0.5	0.5	0.5	0.5	0.5	0.5	0.4	0.4	0.5	0.5	0.5	0.5	0.5	0.4	0.5	0.5	0.5	0.4	0.5	0.4		
CML545	0.6	0.5	0.4	0.4	0.4	0.3	0.5	0.5	0.4	0.5	0.2	0.5	0.5	0.5	0.5	0.4	0.3	0.5	0.6	0.4	0.5	0.4	0.5	0.4	0.5	0.5	0.4	0.4	0.4	0.5	0.4	0.4	
DJ647-22	0.5	0.4	0.5	0.5	0.5	0.4	0.5	0.5	0.6	0.6	0.5	0.5	0.4	0.4	0.4	0.5	0.4	0.4	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.4	0.5	0.5	0.5	0.5	0.4	0.5

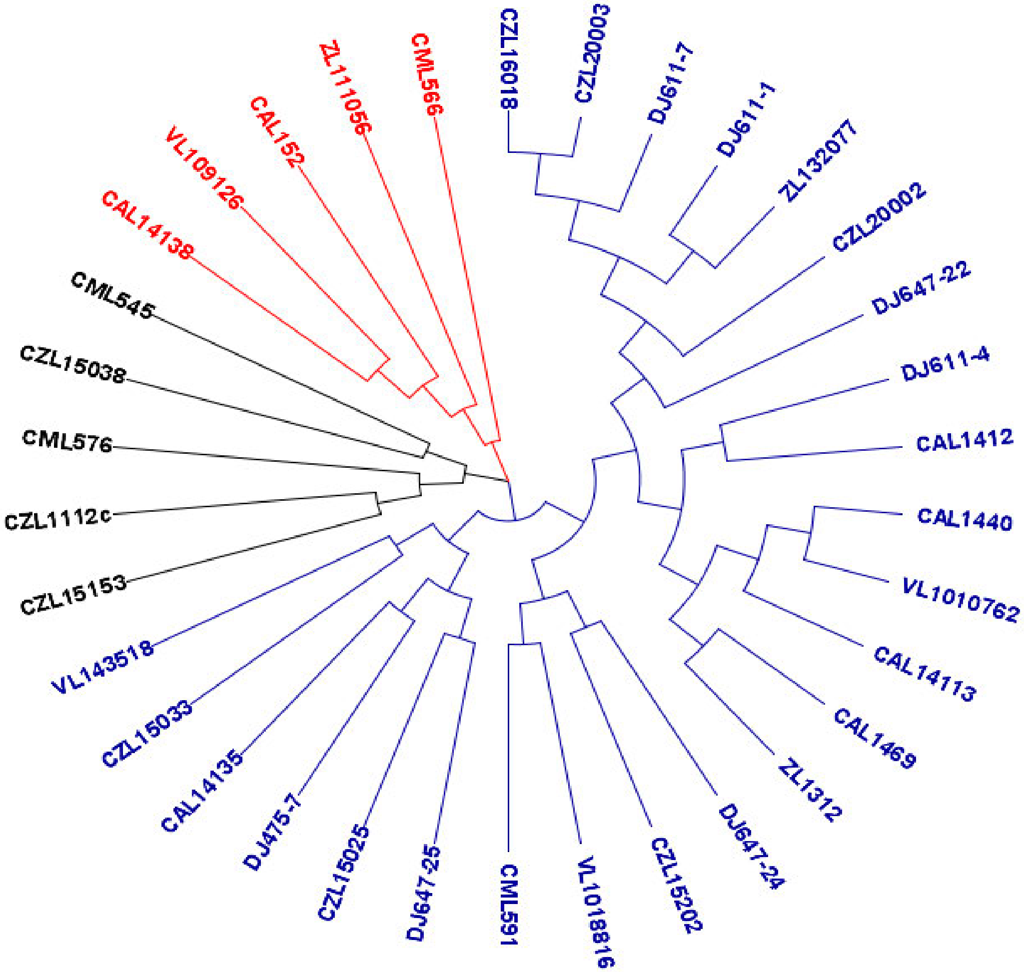


Figure 4.1 Neighbor-joining dendrogram depicting genetic similarities among 35 inbred lines based on identity-by-state genetic distance matrix computed from 93 SNP markers

4.4.3 Genetic purity of heat tolerant donors and CIMMYT-Zimbabwe elite maize inbred lines based on 94 SNPs.

Table 4.4 shows results of genetic purity analysis of the 35 lines genotyped using 94 SNP markers. Of the 35 inbred lines genotyped, 30 (85.76%) lines were genetically pure with homogeneity level ranging from 83% to 100% with an overall mean of 97.4%. In contrast, the other 5 (14.28%) inbred lines including CZL15033 (85.7%), CZL15038 (83%), DJ611-7 (83.7%), CAL440 (93.9%) and CML576 (87.7%) failed the purity test with residual heterozygosity greater than 5% (Table 4.4). A total of 17 of 21 elite lines from CIMMYT-Zimbabwe and 13 of the 14 HSTDLs from CIMMYT-India showed > 95% genetic purity (homogeneity). Of the 19 inbred lines that showed 100% genetic purity, 7 (CAL14113,

CAL1412, CAL14138, CAL1469, CAL152, VL1010762 and ZL11056) were the HTDLs from CIMMYT-India and 12 (CZL16018, DJ647-24, DJ647-25, CZL15153, CZL15202, CZL1112c, DJ475-7, CZL20002, CML591, CML566, CML545 and DJ647-22) were elite inbred lines from CIMMYT-Zimbabwe. Out of 14 exotic HSTDLs evaluated 13 were genetically pure. It is interesting to note that HTDLs were developed through DH method. Missing allele per SNP in this study varied from 0 to 30%, with the overall mean of 9.6% and the percentage of heterozygous alleles ranged from 0 to 17.0 % with a mean of 2.6% (Table 4.4).

Table 4.4 Genetic purity of 14 HSTDLs and 21 CIMMYT-Zimbabwe elite maize inbred lines based on 94 SNPs.

Name	Missing alleles (%)	Heterozygous alleles (%)	Genetic purity (%)
CZL16018	3.2	0.0	100.0
DJ647-24	1.1	0.0	100.0
DJ647-25	0.0	0.0	100.0
CZL15153	1.1	0.0	100.0
CZL15202	0.0	0.0	100.0
CZL1112c	3.2	0.0	100.0
CZL15033	9.7	14.3	85.7
DJ611-1	5.4	1.1	98.9
CZL15025	2.2	1.1	98.9
DJ475-7	22.6	0.0	100.0
CZL15038	5.4	17.0	83.0
DJ611-4	7.5	1.2	98.8
CZL20002	2.2	3.3	96.7
CZL20003	2.2	0.0	100.0
DJ611-7	7.5	16.3	83.7
CAL14113	1.1	0.0	100.0
CAL1412	53.8	0.0	100.0
CAL14135	9.7	1.2	98.8
CAL14138	23.7	0.0	100.0
CAL1440	11.8	6.1	93.9
CAL1469	2.2	0.0	100.0
CAL152	22.6	0.0	100.0
VL1010762	10.8	0.0	100.0
VL1018816	7.5	2.3	97.7
VL109126	8.6	2.4	97.6
VL143518	7.5	1.2	98.8
ZL111056	1.1	0.0	100.0
ZL1312	4.3	2.2	97.8

Name	Missing alleles (%)	Heterozygous alleles (%)	Genetic purity (%)
ZL132077	9.7	4.8	95.2
CML591	4.3	0.0	100.0
CML566	25.8	0.0	100.0
CML576	30.1	12.3	87.7
CML545	4.3	0.0	100.0
DJ647-22	12.9	0.0	100.0
Mean	**9.6%**	**2.6%**	**97.4%**

4.4 Discussion

In any breeding system, genetic diversity is essential to ensures there are many alleles that can be used to develop distinct genotypes that are high yielding and resilient to climate change. However, effective utilization of genetic diversity within germplasm collections requires full understanding of the characteristics of the genetic material. Molecular markers are best used to estimate the genetic diversity of a population because they are independent of the environment. In the current study, 94 SNP markers were used to determine the genetic relatedness and diversity as well as genetic purity of 35 maize inbred lines, comprising of 14 exotic heat tolerant donor lines sourced from CIMMYT-India and 21 elite inbred lines from CIMMYT-Zimbabwe.

The findings of this study revealed that the genetic distance values of the majority (80%) of the genotyped inbred lines fell between 0.4 and 0.6. These results concur with those reported by Josia et al. (2021) who reported 92% of inbred line pairs with genetic distance falling between 0.40 and 0.60. According to a related study by Boakyewaa et al. (2019), genetic distances between 0.32 and 0.42 were found in 67.7% of inbred line pairs. CIMMYT also studied the genetic diversity of 450 inbred lines and discovered that 95% of the pairs of inbred lines had genetic distances between 0.3 and 0.5 (Semagn et al. 2012a). The genetic distance estimate between pairs of inbred lines greater than 0.5 reflects wide genetic diversity (Sserumaga et al. 2019; Badu-Apraku et al. 2021). High genetic distances observed between the studied inbred lines infers that the lines were unique and there is considerable genetic diversity in the germplasm, which might be used to the breeding programme's advantage resulting in high levels of heterosis. Substantial genetic diversity is also important in a breeding programme because it provides the breeder with a wide range of possibilities to consider when breeding for specific objectives. However, Ajala et al. (2019) reported that the information from genetic distance estimation does not guarantee excellent heterosis, therefore, the parents to use in hybrids combinations must exhibit genetic complementarity to produce superior hybrids which can only be determined by making crosses and measuring combining ability.

The higher genetic distance of 0.64 observed between inbred lines pairs CAL14135 and ZL132077, CZL1112c and CZL16018, and CZL15153 and CZL16018 reflect distant relationship, therefore, the CIMMYT-Zimbabwe breeding programme can benefit from the addition of new alleles from these unrelated pairs because they have the potential to provide new alleles. The two pairs of inbred lines from CIMMYT-Zimbabwe that exhibited wide genetic distance and are in different heterotic groups can be used to generate single cross hybrids since high heterosis is possible from genetically diverse inbred lines. The shortest genetic distance of 0.04 observed between heat tolerant donor lines ZL132077 and local line DJ611-1 suggested a close relationship between the lines. These lines are in the same heterotic group, hence, can be used for heat stress pedigree breeding starts.

The neighbor-joining tree clustered inbred lines in three separate groups, implying high level of genetic diversity in the inbred lines assessed in this study. Generally, with a few exceptions, the inbred lines were randomly allocated into different clusters and sub-clusters. Some closely related inbred lines were clustered together or sub-clustered ascertaining the presence of the relationship between the pedigree and the SNP marker grouping in this study. Inbred lines from different clusters have unique alleles that may be useful in breeding for heat tolerance. The genetic diversity observed amongst maize inbred lines in the current study is encouraging to breeders because the diverse inbred lines can be used to generate breeding populations, develop new stress tolerant inbred lines, hybrids and open pollinated varieties. Hallauer e al. (2010) reported that the genetic differences of parental lines determine the heterosis. Therefore, crossing schemes using divergent inbred lines would allow greater success in creating genetic variability (Nyombayire et al. 2016). Additionally, Prasanna (2012) emphasized the importance of effective and reliable selection of inbred lines to facilitate appropriate use of genetic resources in breeding programmes. Subsequently, the relationship among inbred lines that has been observed in this study could be used to design a robust heat tolerant maize breeding programme in Zimbabwe.

Given that the exotic heat tolerant donor lines that were used in the current study already have beneficial traits for tolerance to heat stress, and the local lines have tolerance to drought and diseases stresses, they could be potential candidates for recycling in the breeding programme. Bi-parental crosses among genetically varied inbred lines with good genetic potential would result in combinations of several desirable alleles at several loci, giving rise to new lines with increased yield potential and multiple stress tolerance (Badu-Apraku et al. 2021). Huge genetic diversity gives breeders several possibilities to explore when breeding for specific objectives.

Some inbred lines in the current study were not clustered according to shared pedigree and heterotic grouping, signifying that the selection history of inbred lines derived from the same source population may differ (Boakyewaa et al. 2019). However, some inbred lines have clustered according to their heterotic assemblage (CML545, CML576, CZL15038, CZL1112c and CZL15153). The discrepancies in grouping of inbred lines discovered when comparing molecular results with classification based on pedigree relatedness and heterotic grouping were earlier reported by several authors (Nyombayire et al. 2016; Sserumaga et al. 2019; Boakyewaa et al. 2019; Zawadi et al. 2021; Ayesiga et al. 2023). Genetic drift, selection, and mutation effects may have caused the discrepancies in inbred lines' alignment to heterotic grouping and pedigree history (Wende et al. 2013; Nyombayire et al. 2016).

Keeping the inbred lines' genetic integrity intact is essential for effective hybrid breeding and seed production (Josia et al. 2021). Significant changes in the inbred lines' genetic make-up have an important implication on the quality of products produced. An inbred line is deemed genetically pure or homogeneous if the percentage of heterozygous loci for each SNP analyzed is less than 5% (Semagn et al. 2012a; Gowda et al. 2017). Majority (85.7%) of the lines assessed in this study were genetically pure. These findings align with those of Josia et al. (2021), who observed 67% genetic purity on maize inbred lines genotyped using 92 SNP markers. Instead of being genetically pure with a residual heterozygosity of no more than 5%, 14.24% of the inbred lines employed in this study exhibited residual heterozygosity exceeding 5%. The residual heterozygosity observed in the inbred lines could be due to accidental outcrossing or contamination that can occur during seed production. Human error could also have contributed to genetic impurity of inbred lines such as mistakes made during seed regeneration, maintenance and bulking (Warburton et. al. 2010). These inbred lines need purification by employing the ear-to-row method and appropriate pollination techniques. This also holds true for the inbred line CML576, which has demonstrated 12.7% residual heterozygosity and is used as a parent in certain experimental hybrids.

The nineteen inbred lines that exhibited 100% homozygosity in this evaluation could be linked to the proper maintenance of the inbred lines for multiple generations of selfing. Comparable results were published by Dao (2014) in his investigation utilizing 1237 SNPs, in which most of the inbred lines examined showed 100% homozygosity. The fact that 13 out of 14 HTDLs were genetically pure could be linked to the DH method that was used to develop them. The DH breeding method shorten the breeding cycle and increases efficiency by producing homozygous lines quickly and with greater predictability than those developed through convectional breeding. Majority of the inbred lines employed in this study had greater levels of genetic purity, indicating that the CIMMYT-Zimbabwe maize breeding programme is effective and quality-focused in terms of developing and maintaining inbred lines.

4.5 Conclusion and Recommendations

High genetic distances observed between the pairs of inbred lines demonstrated the distinctiveness of the lines under study as well as the presence of significant genetic variability that may be used in breeding for heat tolerance. The generated information on genetic relatedness and diversity can be utilized as the basis for designing cross combinations by identifying genetically distinct inbred lines to develop superior hybrids and closely related lines to generate breeding populations. The local inbred lines that clustered together with HSTDL (ZL132077 and DJ611-1) and are closely related basing on the genetic distance matrix can be used in developing heat tolerant breeding population. The local inbred lines pairs that showed high genetic distance (CZL1112c and CZL16018, and CZL15153 and CZL16018) can be used in hybrid breeding programme.

Generally, these results show the possibility of running a robust heat stress tolerant maize breeding programme in Zimbabwe and SSA at large. This is because there is genetic diversity among lines some of which have heat stress tolerance to contribute as donors and others as source of local adaptation.

References

Adekemi, S.A.; Menkir, A.; Paterne, A.; Ifie, B.; Tongoona, P.; Unachukwu, N.; Meseka, S.; Mengesha, W.; Gedil, M. Genetic diversity and population structure of maize inbred lines with varying levels of resistance to striga hermonthica using agronomic trait-based and SNP markers. *Plants*. 2020, 9(9), 1-18. doi: 10.3390/plants9091223.

Ajala, S.O.; Olayiwola, M.O.; Ilesanmi, O.J.; Gedil, M.; Job, A.O.; Olaniyan, A.B. Assessment of genetic diversity among low-nitrogen-tolerant early generation maize inbred lines using SNP markers. *South African J. Plant Soil*, 2019. *36*(3), 181-188. https://hdl.handle.net/10520/EJC-168439898f

Ali, M.L.; Rajewski, J.F.; Baenziger, P.S.; Gill, K.S.; Eskridge, K.M.; Dweikat, I. Assessment of genetic diversity and relationship among a collection of US sweet sorghum germplasm by SSR markers. *Molecular Breeding*. 2008, *21*, 497-509. doi: 10.1007/s11032-007-9149-z.

Archana, K.A.; Kuchanur, P.H.; Zaidi, P.H.; Mandal, S.S.; Arunkumar, B.; Patil, A.; Vinayan, M.T; Seetharam, K. Combining ability and heterosis in tropical maize (Zea mays L.) under heat stress. *Journal of Pharmacognosy and Phytochemistry*, 2018, *7*(6), 1026-1031.

Ayesiga, S.B.; Rubaihayo, P.; Oloka, B.M.; Dramadri, I.O.; Edema, R.; Sserumaga, J.P. Genetic Variation Among Tropical Maize Inbred Lines from NARS and CGIAR Breeding Programs. *Plant Mol. Biol. Report.* 2023, *41*(2), 209-217. doi: 10.1007/s11105-022-01358-2.

Badu-Apraku, B.; Garcia-Oliveira, A.L.; Petroli, C.D.; Hearne, S.; Adewale, S.A.; Gedil, M. Genetic diversity and population structure of early and extra-early maturing maize germplasm adapted to sub-Saharan Africa. *BMC Plant Biol.*, 2021. *21(1),* 1-15. doi: 10.1186/s12870-021-02829-6.

Boakyewaa, A.G.; Badu-Apraku, B.; Akromah,R.; Garcia-Oliveira, A.L.; Awuku, F.J.; Gedil,M. Genetic diversity and population structure of early-maturing tropical maize inbred lines using SNP markers. PloS one. 2019; 14(4), e0214810. https://doi.org/10.1371/journal.pone.0214810 PMID: 3096489

Bradbury, P.J.; Zhang, Z.; Kroon, D.E.; Casstevens, T.M.; Ramdoss, Y.; Buckler, E.S. TASSEL:

software for association mapping of complex traits in diverse samples. *Bioinformatics*, 2007. *23*(19), 2633-2635. doi: 10.1093/bioinformatics/btm308.

Cairns, J.E.; Sonder, K.; Zaidi, P.H.; Verhulst, N.; Mahuku, G.; Babu, R.; Nair, S.K.; Das, B.; Govaerts, B.; Vinayan, M.T.; Rashid, Z. Maize production in a changing climate: impacts, adaptation, and mitigation strategies. *Adv. Agron.* 2012, *114*, 1-58: doi: 10.1016/B978-0-12-394275-3.00006-7.

Dao, A.; Sanou, J.; Mitchell, S.E.; Gracen, V.; Danquah, E.Y. Genetic diversity among INERA maize inbred lines with single nucleotide polymorphism (SNP) markers and their relationship with CIMMYT, IITA, and temperate lines. *BMC genetics*, 2014. *15*, 1-14. doi: 10.1186/s12863-014-0127-2.

Deryng, D.; Conway, D.; Ramankutty, N.; Price, J.; and Warren, R. Global crop yield response to extreme heat stress under multiple climate change futures. *Environ. Res. Lett.* 2014. *9*(3), 034011. doi: 10.1088/1748-9326/9/3/034011.

Ertiro, B.T.; Ogugo, V.; Worku, M.; Das, B.; Olsen, M.; Labuschagne, M.; Semagn, K. Comparison of Kompetitive Allele Specific PCR (KASP) and genotyping by sequencing (GBS) for quality control analysis in maize. *BMC genomics*. 2015. 908 (*16),* 1-12. DOIhttps://doi.org/10.1186/s12864-015-2180-2

Fernandez, E.C.J.; Nuñez, J.P.P.; Gardoce, R.R.; Manohar, A.N.C.; Bajaro, R.M.; Lantican, D.V. Genetic purity and diversity assessment of parental corn inbred lines using SSR markers for Philippine hybrid breeding. *SABRAO J. Breed. Genet,* 2023. *55*(3), 598-608. http://doi.org/10.54910/sabrao2023.55.3.1.

Govindaraj, M.; Vetriventhan, M.; Srinivasan, M. Importance of genetic diversity assessment in crop plants and its recent advances: an overview of its analytical perspectives. *Genet. Res. Int.* 2015, 1-14. doi: 10.1155/2015/431487.

Gowda, M.; Worku, M.; Nair, S.K. Quality Assurance / Quality Control (QA / QC) in Maize Breeding and Seed Production: Theory and Practice. 2017 CIMMYT.

Hallauer, A. R.; Carena, M.J.; Miranda Filho, J.D. Quantitative genetics in maize breeding (Vol. 6). Springer Science & Business Media. 2010.

Huma, B.; Hussain, M.; Ning, C.; Yuesuo, Y. Human benefits from maize. *Sch. J. Appl. Sci. Res*, 2019, *2*(2), 4-7.

Jodage, K.; Kuchanur, P.H.; Zaidi, P.H.; Patil, A.; Seetharam, K.; Vinayan, M.T.; Arunkumar, B. Genetic analysis of heat stress tolerance and association of traits in tropical maize (*Zea mays* L.). *Environ. Ecol.* 2017, *35*(3C), 2354-2360:

Jodage, K.; Kuchanur, P.H.; Zaidi, P.H.; Patil, A.; Seetharam, K.; Vinayan, M.T.; Arunkumar, B. Genetic analysis of heat adaptive traits in tropical maize (*Zea mays* L.). *Int. J. Curr. Microbiol. Appl. Sci.* 2018, 7(1), 3237–3246. doi: 10.20546/ijcmas.2018.701.387.

Josia, C.; Mashingaidze, K.; Amelework, A.B.; Kondwakwenda, A.; Musvosvi, C.; Sibiya, J. SNP-based assessment of genetic purity and diversity in maize hybrid breeding. *PLoS One*, 2021.*16*(8), 1-14. doi: 10.1371/journal.pone.0249505.

Khodarahmpour, Z.; Choukan, R.; Bihamta, M.R.; Majidi, H.E. Determination of the best heat stress tolerance indices in maize (*Zea mays L.*) inbred lines and hybrids under Khuzestan province conditions. *J. Agric. Sci. Technol.* 2011, 13(1), 111–121.

Mafakheri, K.; Bihamta, M.R.; Abbasi, A.R. Assessment of genetic diversity in cowpea (Vigna unguiculata L.) germplasm using morphological and molecular characterisation. *Cogent Food Agric.*, 2017.*3*(1), 1–20. doi: 10.1080/23311932.2017.1327092.Matsuoka, Y.; Vigouroux, Y.; Goodman, M.M.; Sanchez G, J.; Buckler, E.; Doebley, J.; A single domestication for maize shown by multilocus microsatellite genotyping. *Proc. Natl. Acad. Sci.* 2002. *99*(9), 6080–6084. doi: 10.1073/pnas.052125199.

Mukaro, R.; Kamutando, C.N.; Magorokosho, C.; Mutari, B.; Zaidi, P.H.; Kutywayo, D.; Sibiya, J. Genetic potential of tropically adapted exotic maize (*Zea mays* L.) heat-tolerant donor lines in sub-tropical breeding programs. *J. Agron*, 2023. *13*(8), 1-6: https://doi.org/10.3390/agronomy13082050

Mushtaq, M.; Bhat, MA.; Bhat, J.A.; Mukhtar, S.; Shah, A.A. Comparative analysis of genetic diversity of maize inbred lines from Kashmir Valley using agro-morphological and SSR markers. *SABRAO J. Breed. Genet.* 2016, 48(4),518–527

Nadeem, M.A.; Nawaz, M.A.; Shahid, M.Q.; Doğan, Y.; Comertpay, G.; Yıldız, M.; Hatipoğlu, R.; Ahmad, F.; Alsaleh, A.; Labhane, N.; Özkan, H. DNA molecular markers in plant breeding: Current status and recent advancements in genomic selection and genome editing. *Biotechnol. Biotechnol. Equip.* 2018. 32(2): 261–285. doi: 10.1080/13102818.2017.1400401.

Nelimor, C.; Badu-Apraku, B.; Tetteh, A.Y.; N'guetta, A.S. Assessment of genetic diversity for drought, heat and combined drought and heat stress tolerance in early maturing maize landraces. *Plants*. 2019.*8*(11), 1-9: doi: 10.3390/plants8110518.

Nyombayire, A.; Derera, J.; Sibiya, J.; Gasura, E.; Ngaboyisonga, C. Genetic diversity among maize inbred lines selected for the mid-altitudes and highlands of Rwanda. *Maydica*. 2016. *61*(2), 7-13.

Prasanna, B.M. Diversity in global maize germplasm: Characterization and utilization. J. Biosci. 2012. 37(5): 843–855. doi: 10.1007/s12038-012-9227-1.

Semagn, K.; Beyene, Y.; Makumbi, D.; Mugo, S.; Prasanna, B.M.; Magorokosho, C.; Atlin, G. Quality control genotyping for assessment of genetic identity and purity in diverse tropical maize inbred lines. *Theor. Appl. Genet.*, 2012a. *125(7): 1487–1501. doi: 10.1007/s00122-012-1928-1.*

Semagn, K.; Magorokosho, C.; Vivek, B.S.; Makumbi, D.; Beyene, Y.; Mugo, S.; Prasanna, B.M.; Warburton, M.L.; Molecular characterization of diverse CIMMYT maize inbred lines from eastern and southern Africa using single nucleotide polymorphic markers. *BMC Genomics*, 2012b. 13(1). doi: 10.1186/1471-2164-13-113.

Silva, K.J.; Guimarães, C.T.; Guilhen, J.H.S.; Guimarães, P.E.D.O.; Parentoni, S.N.; Trindade, R.D.S.; de Oliveira, A.A.; Bernardino, K.D.C.; Pinto, M.D.O.; Dias, K.O.D.G.; Bernardes, C.D.O. High-density SNP-based genetic diversity, and heterotic patterns of tropical maize breeding lines. *Crop Science*, 2020. 60(2): 779–787. doi: 10.1002/csc2.20018.

Sserumaga, J.P.; Makumbi, D.; Warburton, M.L.; Opiyo, S.O.; Asea, G.; Muwonge, A.; Kasozi, C.L. Genetic diversity among tropical Pro-vitamin A maize inbred lines and implications for a biofortification program. *Cereal Res. Commun.* 2019. 47(1), 134–144. doi:

10.1556/0806.46.2018.066.

Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; and Kumar, S. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* 2013. 30(12), 2725–2729. doi: 10.1093/molbev/mst197.

Warburton ML, Setimela P, Franco J, Cordova H, Pixley K, Ba¨nziger M et al. Toward a cost-effective fingerprinting methodology to distinguish maize open-pollinated varieties. Crop Science. 2010; 50:467– 477

Wende, A.; Shimelis, H.; Derera, J.; Mosisa, W.; Danson, J.; Laing, M.D. Genetic interrelationships among medium to late maturing tropical maize inbred lines using selected SSR markers. *Euphytica*, 2013. 191(2): 269–277. doi: 10.1007/s10681-012-0826-6.

Zawadi, M.; Sibiya, J.; Mashingaidze, K.; Amelework, A.B.; Kondwakwenda, A.; Musvosvi, C. Molecular characterization and diversity analysis of selected maize inbred lines using single-nucleotide polymorphism markers. *Can. J. Plant Sci.*, 2021.*101*(2), pp.240-248.

CHAPTER 5:

Grain yield stability of early maturing maize hybrids under heat stress prone agro-ecologies of Zimbabwe

Abstract

Heat stress is one of the main abiotic stressors limiting maize (Zea mays *L.*) productivity in sub Saharan Africa (SSA). In this study, 25 hybrids (20 early maturing commercial hybrids + 5 experimental hybrids selected for heat stress adaptation), were evaluated across 6 locations during the 2020/21-2021/22 summer seasons, and were at each location, established using a 5 x 5 square lattice design, replicated three times. The trials were conducted to: (i) identify commercial hybrids adapted to heat stress (HTS) and random drought and heat stress (RDHS); and (ii) identify commercial hybrids with stable and superior grain yield performance across stress and non-stress conditions. Highly significant ($P < 0.001$) differences were exhibited by hybrids on grain yield and the hybrid x location x year interaction effect on grain yield was also significant. Commercial hybrids G3 (4.79 t ha^{-1}), G20 (3.99 t ha^{-1}) and G22 (4.09 t ha^{-1}) as well as experimental hybrid, G4 (4.31 t ha^{-1}), were the most adapted under heat stress conditions. Under RDHS conditions, the most adapted commercial genotypes were G12 (4.66 t ha^{-1}), G14 (4.39 t ha^{-1}), G15 (4.02 t ha^{-1}), G13 (4.17 t ha^{-1}), G21 (3.76 t ha^{-1}) and G25 (3.50 t ha^{-1}), with G12 being the most adapted under this agro-ecology. The 'ranking' genotype-genotype x environment (GGE) biplot identified commercial hybrids G16 (4.94 t ha^{-1}) and G3 (4.79 t ha^{-1}) as high yielding and stable across stress (HTS and RDHS) and non-stress conditions. The experimental hybrid G6 (4.77 t ha^{-1}) and G7 (4.67 t ha^{-1}) were stable across environments. The experimental genotype, G8 (5.47 t ha^{-1}), was overall, the highest performing but was unstable, therefore can be recommended for specific adaptation. Overall, data demonstrated potential of the already released maize varieties in aiding maize productivity under heat and combined heat and drought stress conditions in SSA. Furthermore, the productive experimental genotypes selected for heat stress adaptation, can be helpful in complementing the adapted commercial varieties under the defined stress prone agro-ecologies.

Key words: Heat stress, maize, stability analysis, genotype x environment interaction

5.1 Introduction

Maize (*Zea mays* L.) is an important and widely grown cereal crop in the world (Prasanna et al. 2021). The demand for maize is projected to double by 2050, making it the most popular food crop with the highest production globally (Rosegrant et al. 2009). Grown mostly by smallholder farmers in varied environmental conditions, maize is primarily a rain-fed crop in Sub-Saharan Africa (SSA) (Nelimor et al. 2019). Every year, more than 38 million metric tonnes of maize are produced to feed and support more than 300 million families in SSA (Nyaga et al. 2019). In addition to providing smallholder farmers with a significant source of income, maize makes up 15- 20% of the average daily caloric intake of the people in the SSA (FAOSTAT, 2021; Kolawole et al. 2021). In Zimbabwe, maize is the most important crop in terms of production area and food security, according to a participatory rural assessment study conducted by Mutari et al. (2021).

In spite of its significance in SSA, maize production and productivity are hindered by several biotic, abiotic, and socio-economic factors, leading to notable reductions in grain yields. (Ali et al. 2015; Nyaga et al. 2019). Among the abiotic factors, stresses brought on by climate change are posing the biggest worldwide threat to sustainable agricultural production (Rahman et al. 2015). In SSA, global warming has altered weather patterns, resulting in unpredictable rainfall patterns and extremely high temperatures (Rosegrant et al. 2009; Rahman et al. 2015). Heat stress brought on by high ambient temperatures has been frequently reported as the primary factor causing maize grain production instability, even though drought and heat stress typically occur simultaneously (Ali et al. 2015; Akter and Rafiqul, 2017; Tiwari and Yadav, 2019; Koirala et al. 2021; Yousaf et al. 2022). Crops usually experience physiological drought under heat stress conditions and this relatively explains high yield penalties in comparison with drought stress (Thayil et al. 2020). Additionally, drought stress lowers the crop's chlorophyll concentration, but to a lesser extent than heat stress (Lamaoui et al. 2018). In addition to all these reports, climate projections reflects continuous rise in temperature which leads to lengthy heat stress periods and significant grain yield reduction in maize (Intergovernmental Panel on Climate change, 2016; Pavani et al. 2019). Simulation studies on maize for heat stress conducted at Chiredzi and Matopos Research stations in Zimbabwe revealed that a 1, 2 and 4 °C rise in mean air temperature in relation to the baseline climate, results in a yield drop of 1–21%, 3–34% and 17–67%, respectively (Tesfaye et al. 2018).

Several researchers have reported significant yield losses due to heat stress (Fonseca and Westgate, 2005; Lobell et al. 2012; Hasanuzzaman et al. 2013; Meseka et al. 2018). Monsanto (2012) reported that the quantity of maize grain yield lost as a result of heat stress varied from 15% to 40% and might even reach 70% based on the timing, length, and intensity of the stress. For every 1°C rise in temperature above 29^{o}C, Brown et al. (2011) reported a global maize grain yield drop of 10%. Similarly, Thomson (1966) reported a 10% yield loss in the United States of America Corn Belt due to rise in temperature from 22 to 28°C during the grain filling phase. Badu-Apraku et al. (1983) revealed a 42% yield reduction in grain yield due to mean daily temperature increase by 6°C. Koirala et al. (2017) observed that heat stress in Nepal caused a 75% decrease in maize grain yield in the spring and early summer. Grain yield reduction of 101 kgha^{-1}day^{-1} has been reported when temperatures are kept above 35^{0}C during the pollination and grain filling phase (Rahman et al. 2013; Naveed et al. 2016). Based on the results of more than 20,000 historical maize trial data from southern Africa, a linear decline in maize yield was observed with each cumulative degree day above 30 °C (Lobell et al. 2011).These results show the urgent need for adaptive measures to cope with deleterious effects of HTS.

Among the HTS mitigation strategies, genetic resistance was reported to be the most sustainable and cost-effective (Cairns et al. 2012; Meseka et al. 2018). However, breeding maize for heat stress tolerance in SSA is still in its early stages and needs greater attention than it has received in the past (Cairns et al. 2012; Gazala et al. 2019). Furthermore, the time lag between development of a variety up to adoption by farmers is long (Cairns et al. 2013). Hence, there is need to screen the existing genotypes (commercial varieties) for heat stress tolerance, in order to identify adapted genotypes that can immediately be recommended for production in heat stress prone agro-ecologies in SSA. The genotype performance in multi-locational evaluations is influenced by genetics, the production environment, and the interplay between genotype and environment. (Oliveira et al. 2014; Matova et al. 2022). Genotype x environment interaction (GEI) is usually high under stress environments causing inconsistences in genotype ranking and complicating the identification of superior genotypes (Mebratu et al. 2019; Bocianowski et al. 2019; Rezende et al. 2020). Measuring and interpreting GEI helps to optimize breeding strategies for selecting genotypes that are specifically or widely adapted to diverse environmental conditions (Badu-Apraku et al. 2010; Workie et al. 2013). Among the statistical models that have been used to analyze GEI, the genotype, genotype x environment (GGE) biplot is one of the most effective (Couto et al. 2015). It is used to identify genotypes that are stable and high yielding, identify environments with representativeness and discriminating ability, and determine genotype that performs best in each environment (which-won-where patterns) (Yan and Tinker, 2006; Couto et al. 2015).

The current investigation was conducted to evaluate early maturing commercial hybrids currently on the market in Zimbabwe and heat stress experimental hybrids under heat stress (HTS), random drought and heat stress (RDHS), and optimal conditions (OC). The study was aimed at (i) identify commercial and experimental hybrids adapted under HTS as well as RDHS conditions; and (ii) identify commercial and experimental hybrids that exhibit consistent and improved grain yield performance under both stress and non-stress conditions. We hypothesize that, among the early maturing maize hybrids already released for cultivation in Zimbabwe, which were not specifically bred for heat and combined drought and heat stress adaptation, hybrids adapted and productive under these stress factors exist. In addition, the commercial hybrids adapted and productive under stress can be complemented by maize experimental hybrids specifically developed for heat stress adaptation for enhancement of maize productivity under stress conditions in SSA.

5.2 Materials and methods

5.2.1 Description of the germplasm

A total of 25 maize hybrids, comprising of 20 early maturing commercial hybrids that are currently on the market in Zimbabwe and 5 experimental hybrids selected for heat stress adaptation (Table 5.1), were evaluated in six locations in Zimbabwe over two years in summer and winter during 2020/2021 and 2021/2022 seasons. The commercial hybrids were sourced from local seed companies in Zimbabwe, and these had not been specifically bred for heat stress tolerance. In contrast, the experimental hybrids obtained from CIMMYT-Zimbabwe (the checks), were selected for heat stress adaptation. Table 5.1 shows the genotypes that were used, their origin and the year of release.

Table 5.1 Early maturing commercial hybrids and heat stress tolerant experimental hybrids evaluated under stress and non-stress conditions during the 2020/21 and 2021/22 summer and winter seasons in Zimbabwe.

Genotype	Genotype Code	Source	Year of release
G1	PGS1	Progene seeds	2015
G2	PGS2	Progene seeds	2008
G3	PGS3	Progene seeds	2015
G4	CIMMYTexp1	CIMMYT-Zimbabwe	Experimental
G5	CIMMYTexp2	CIMMYT-Zimbabwe	Experimental
G6	CIMMYTexp3	CIMMYT-Zimbabwe	Experimental
G7	CIMMYTexp4	CIMMYT-Zimbabwe	Experimental
G8	CIMMYTexp5	CIMMYT-Zimbabwe	Experimental
G9	AGR1	Agriseeds	2008
G10	MKS1	Mukushi Seeds	2014
G11	MKS2	Mukushi seeds	2015
G12	PAN1	Pannar	2003
G13	PAN2	Pannar	2007
G14	PNR1	Pioneer	2013
G15	PNR2	Pioneer	2014
G16	SC1	Seed-Co	2019
G17	SC2	Seed-Co	1999
G18	SC3	Seed-Co	2015
G19	SC4	Seed-Co	1998
G20	SC5	Seed-Co	2012
G21	SC6	Seed-Co	2012
G22	SYNG1	Syngenta	2017
G23	SVAN1	Savannah seeds	2011
G24	BYR1	Bayer Zimbabwe Pvt Ltd.	2012
G25	BYR2	Bayer Zimbabwe Pvt Ltd.	2012

5.2.2 Experimental sites

The trials were established across six sites (Table 5.2) under three management regimes including managed heat stress (HTS), random drought and heat stress (RDHS), and optimal condition (OC; well-watered and well fertilized). Briefly, the RDHS trials were planted during the rainy (main) season without supplementary irrigation in areas traditionally known to be prone to drought and heat. On the other hand, OC trials were also conducted during the rainy season but with supplementary irrigation. Managed heat stress trials were planted during the off-season entirely under irrigation. In order to induce heat stress, planting was done at the end of July so that the flowering period known to be the most sensitive to heat stress (Zaidi et al. 2016) synchronized with the highest temperatures that occur in September and October in Zimbabwe (Intergovernmental Panel on Climate change, 2016). Specific planting dates were thus the 29th and 30th of July during 2020/21 and 2021/22 seasons, respectively. Weather data (that is, maximum and minimum temperature, relative humidity) recorded at Chiredzi research station during winter period is highlighted in Table 5.3.

5.2.3 Experimental design and field management

The 25 hybrids were planted in a 5 x 5 square lattice design (Patterson and Williams, 1976) replicated thrice at each site. Each plot was planted on two rows of 4 m length, with inter-row spacing of 0.75 m and intra-row spacing of 0.25 m. Initially, two seeds were planted per station and later thinned to one at two-leaf stage to attain an optimum plant population of 53,333 plants ha^{-1}. Compound D (NPK 7:14:7) basal fertilizer was applied at a rate of 300 kg/ha at planting and ammonium nitrate (34.5% N) was split applied at 4 and 8 weeks after planting as top dressing at a rate of 150 kg ha^{-1} per application to achieve 300 kg ha^{-1}. Before planting, 100 ml ha^{-1} of Karate (Lambdacyhalomethrin) was applied to control termites. Control of the fall armyworm (*Spodoptera frugiperda*) and the maize stem borer (*Busseola fusca*) was achieved by applying 250 ml ha^{-1} of emamectin benzoate and acetamiprid to the crop. Pre-emergence herbicides (metolachlor) and post-emergence herbicides (stellar star and paraqat) were used to keep the experimental plots free of weeds, and hand weeding was subsequently done when necessary.

Table 5.2 Characteristics of the sites used during 2020/21 and 2021/22 seasons

Location	Management	Altitude (masl)	Latitude	Longitude	Soil type	Average rainfall (mm)
CRS	Managed heat stress	409	$21^{0}010$ S	21˚250 E	Red clay	Off-season
CRS	Optimal	409	$21^{0}010$ S	21˚250 E	Red clay	<500
HRS	Optimal	1506	$17^{0}480$ S	31˚030 E	Red clay	<1000
KRS	RDHS	1149	$18^{0}940$ S	29˚250 E	Red clay	700-1000
MRS	RDHS	1338	$20^{0}384$ S	$28^{0}507$E	Black clay	500-750
PES	RDHS	962	17°049 S	31°673E	Red clay	700-1000

CRS = Chiredzi Research Station, HRS = Harare Research Station, KRS = Kadoma Research Station, MRS = Matopos Research Station, PES = Panmure Experiment Station, RDHS = Random drought and heat stress, masl = meters above sea level

Table 5.3 Monthly average weather records for Chiredzi Research station during the winter period (2020/21 and 2021/22)

	2020/2021 season			2021/2022 s	
Months	Max(˚C)	Min(˚C)	RH (%)	Max(˚C)	Min(˚C)
July	26.02	11.72	52.04	26.53	13.62
August	29.8	14.33	50.5	30.43	14.37
Sept	37.55	20.87	46.78	32.53	16.6
October	35.62	19.42	49.82	36.66	20.77
November	36.36	21.86	49.9	33.06	20.78
December	34.44	22.29	61.02	34.56	21.31

Max= maximum temperature; Min=Minimum temperature; RH= relative humidity

5.2.4 Data collection and analysis

At each site, the following agronomic traits were recorded per plot: (i) anthesis date (AD; estimated as the number of days after planting when 50% plants within the plot shed pollen), (ii) silking date (SD; calculated as number of days after planting when 50% of the plants in each plot produce silk), (iii) anthesis-silking interval (ASI; estimated as the difference between days to 50% silking and 50% anthesis), and (iv) grain yield (GYD: measured in kg/plot and adjusted to 12.5% moisture content). These traits were recorded as described by Magorokosho et al. (2009)**.** The homogeneity of error variances for the evaluated traits in each environment was tested using the Bartlett's test and the normality was tested using the Shapiro Wilk test. The results showed that the residual variance for grain yield was heterogeneous, hence the residuals' normality was improved by performing a square root transformation (Malato, 2023).

Analysis of variance (ANOVA) for grain yield, anthesis date and anthesis silking interval within and across environments was performed using restricted maximum likelihood (REML) in Genstat® Discovery 18th Edition (Payne et al. 2018). The residual effect, genotypic effect, and its interactions with environments (genotype x location, genotype x year, genotype x year x environment) were treated as fixed effects (Yan, 2021) and the environment main effects (location, year, location x year), replication and block were treated as fixed effects (Yan, 2021). The following model was used:

$$Y_{ijklm} = \mu + G_i + L_j + Y_k + Rl_{(jk)} + Bm_{(jkl)} + GL_{ij} + GY_{ik} + LY_{jk} + GLY_{ijk} + e_{ijklm} \quad (1)$$

where Y_{ijklm} is the response of the i^{th} genotype in j^{th} location and lth replication within year and m^{th} block within location, μ is the grand mean, Gi is the random effect of the ith genotype, L_j is the fixed effect of the j^{th} location, Y_k is the fixed effect of the year k, $Rl_{(jk)}$ is the fixed effect of the replicate l nested within location j in year k, $Bm_{(jkl)}$is the fixed effect of the block m nested within location j in year k and replicate l, GL_{ij} is the random effect of the interaction between genotype i and location j, GY_{ik} is the random effect of the interaction between genotype i and year k, LY_{jk} is the random effect of the interaction between location j and year k, and GLY_{ijk} is the random effect of genotype by location by year interaction, and e_{ijlkm} is the random error.

Because the GEI was significant for GYD, stability and adaptability analysis of the genotypes was done. A genotype main effect plus genotype x environment interaction (GGE) biplot analysis was performed based on principal component analysis (PCA) of environment-centered data to analyze the variance resulting from genotypes and genotype x environment. Based on the PCA of environment-centered data, the genotype main effect plus genotype x environment interaction (GGE) biplot analysis was done to dissect the variation caused by genotypes and genotype x environment (Yan et al. 2001). The GGE biplot was generated using Genstat 18th edition software (Payne et al. 2018) using the model based on singular value decomposition (SVD) of the first two principal components according to Yan et al. (2001) as follows:

$$Y_{ij} - \mu - \beta_j = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_i 2 \, \eta_{j2} + \varepsilon_{ij} \qquad (2)$$

where: Y_{ij} is the the yield mean of ith hybrid in jth environment, μ is the the grand mean, β_j is thethe main effect of environment j, $\mu + \beta_j$is the mean yield across all hybrids in environment j, λ_1 and λ_2 is the singular values (SV) for the first and second principal component (PC1 and PC2), respectively, ξ_{i1} and ξ_i2 is the eigen vectors of hybrid i for PC1 and PC2, respectively, $\eta_{j1}$and η_{j2} is the eigen vectors of environment j for PC1 and PC2, respectively and ε_{ij} is the residual associated with hybrid i in environment j.

The broad-sense heritability (H^2) for the traits was estimated for different management conditions and across environments using variance components following the method suggested by Hallauer et al. (2010) as follows:

$$H^2 = \frac{\sigma^2 G}{\sigma^2 G + \frac{\sigma^2 GxL}{E} + \frac{\sigma^2 E}{ER}}$$

where σ^2G = genotypic variance, σ^2GxL = genotype x location variance, σ^2E = residual variance, E = number of environments, R= number of replications

5.3 Results

5.3.1 Agronomic performance of the commercial hybrids under stress and non-stress conditions

Table 5.4 shows the results of the combined analysis of variance (ANOVA) for GYD, AD, and ASI measured during 2020/21 and 2021/22 seasons. The ANOVA across locations and years under HTS, RDHS and OC revealed significant ($P<0.05$) differences among hybrids as well as hybrids x year x location interaction on GYD, AD and ASI. The effect of the year was significant ($P < 0.001$) on GYD and ASI but no effect was observed on AD. On the other hand, significant ($P < 0.001$) years x location interaction effect were observed on all the agronomic traits that were measured (Table 5.4).

Table 5.4 Combined ANOVA for GYD, AD and ASI measured in trials conducted during the 2020/21 and 2021/22 seasons in Zimbabwe.

		GYD (kg ha^{-1})		AD (days)		ASI (days)	
Source of variation	DF	SS	MS	SS	MS	SS	MS
Year	1	92.87	92.87***	5.92	5.92^{ns}	38.028	38.03***
Location (Year)	10	1153.28	115.33***	8363.45	836.35***	737.869	73.79***
Replication (Location x Year)	24	63.47	2.65***	272.8	11.37^{ns}	66.853	2.79**
Hybrid	24	260.63	10.86***	2402.15	100.09***	66.278	2.76**
Hybrid x Year	24	27.27	1.14^{ns}	365.38	15.22^{ns}	33.722	1.41*
Hybrid x Location	120	283.28	2.36***	1542.53	12.85^{ns}	190.776	1.59**
Hybrid x Location x Year	120	347.93	2.90***	3038.63	25.32***	292.744	2.44**
Residual	576	741.64	1.29	6251.87	10.85	782.48	1.358
Total	899	2970.38	3.3	22242.73	24.74	2208.75	2.457

$^{*, **, ***}$ indicate mean squares significant at $P \le 0.05$, $P \le 0.01$ and $P \le 0.001$ probability levels, respectively, ns = not significant

Table 5.5 shows the mean GYD, AD and ASI of the hybrids measured under HTS, RDHS, OC and across these management conditions. The mean GYD of the hybrids under HTS was 2.42 tha^{-1} with yield ranging between 1.71 t ha^{-1} and 3.48 t ha^{-1}. High yielding genotypes under heat stress were G4 (3.48 t ha^{-1}), G6 (3.2 t ha^{-1}), G16 (3.02 t ha^{-1}), G3 (3.0 t ha^{-1}) and G7 (2.19 t ha^{-1}). The commercial varieties, G16 and G3, outperformed the three experimental check hybrids, i.e., G7 (2.19 t ha^{-1}), G8 (2.88 t ha^{-1}) and G5 (2.30 t ha^{-1}), under HTS conditions. The number of days to pollen shedding ranged from 51 to 61 and ASI ranged from 0 to 3 days under HTS conditions. Under RDHS conditions, GYD of the hybrids ranged from 2.93 t ha^{-1} to 5.27 t ha^{-1} with a mean of 3.9 t ha^{-1}, whereas G8 (5.27 t ha^{-1}), G16 (4.76 t ha^{-1}), G12 (4.59 t ha^{-1}), G7 (4.40 t ha^{-1}) and G6 (4.38 t ha^{-1}) were the highest yielders. Two commercial hybrids G16 (4.76 t ha^{-1}) and G12 (4.59 t ha^{-1}), performed better than four experimental hybrids, i.e., G7 (4.40 t ha^{-1}), G6 (4.38 t ha^{-1}), G4 (3.91 t ha^{-1}) and G5 (4.12 t ha^{-1}) under RDHS. The AD ranged from 57 to 63 days and the ASI ranged from 0 to 2 days under RDHS conditions. Under OC, mean GYD was 5.22 t ha^{-1} with GYD ranging from 3.90 t ha^{-1} to 7.08 t ha^{-1}. Commercial hybrids G3 (6.61 t ha^{-1}), G16 (6.16 t ha^{-1}), and G12 (6.10 t ha^{-1}) performed better than the experimental hybrids G4 (5.31 t ha^{-1}), G5 (5.96 t ha^{-1}), and G7 (4.40 t ha^{-1}). However, all the hybrids were outperformed by the experimental hybrid G8 (7.08 t ha^{-1}). The AD ranged from 59 to 66 days and the ASI ranged from 0 to 1.4 days under OC conditions (Table 5.5).

Across environments, GYD ranged from 3.1 t ha^{-1} to 5.47 t ha-^{1}, with an overall mean of 4.05 t ha^{-1}. The two highest yielding commercial hybrids were G16 (4.94 t ha^{-1}) and G3 (4.79 t ha^{-1}), which performed better than experimental hybrids G6 (4.77 t ha^{-1}) and G7 (4.67 t ha^{-1}), G4 (4.31 t ha^{-1}), G5 (4.42 t ha^{-1}). However, experimental hybrid G8 (5.47 t ha^{-1}) performed better than all the commercial and experimental hybrids. It is interesting to note that all the experimental hybrids were among the top 10 best yielders across environments Table 5.5. Across environments, the anthesis dates ranged from 56 to 63days whereas ASI ranged between 0 to 2 days (Table 5.5).

Heritability estimates for GYD under HTS, RDHS, OC and across environments were 17%, 45%, 35% and 67% respectively. Heritability of zero percent was recorded on ASI under OC and RDHS environments and for AD under HTS. On the other hand, heritability estimates observed for AD under RDHS and OC conditions were 70% and 71%, respectively, while AD across environments had heritability estimate of 83% (Table 5.5).

Table 5.5 Commercial and experimental hybrids adapted to heat, random drought and heat and optimal condition

		Across				Managed Heat				Random heat and drought				Optimal management			
Entry	Entry name	GYD	Rank	AD	ASI	GYD	Rank	AD	ASI	GYD	Rank	AD	ASI	GYD	Rank	AD	ASI
G1	PGS1	3.77^{g-k}	16	62.67^{a-d}	0.75^{ef}	2.02^{e-h}	21	56.63	0.5	3.39^{f-j}	18	63.3^{ab}	1.2^{c-e}	5.21^{c-i}	14	65.3^{ab}	0.2^{d-f}
G2	PGS2	3.82^{f-j}	14	60.62^{e-h}	1.22^{b-2}	1.71^{h}	25	54.59	1.65	3.48^{e-j}	17	61.1^{d-g}	1.2^{c-e}	5.39^{c-h}	11	62.5^{f-j}	0.6^{b-f}
G3	PGS3	4.79^{bc}	3	62.85^{a-c}	1.15^{c-f}	3.00^{a-c}	4	57.02	1.32	4.17^{b-e}	6	63.7^{a}	1.5^{a-d}	6.61^{ab}	2	64.5^{b-d}	0.3^{c-f}
G4	CIMMYTexp 1	4.31^{c-f}	9	58.5^{kl}	1.65^{a-c}	3.48^{a}	1	52.16	1.17	3.91^{c-g}	10	58.8^{h}	2.1^{a}	5.31^{c-i}	13	61.3^{jk}	0.9^{a-d}
G5	CIMMYTexp 2	4.42^{b-e}	7	60.3^{g-i}	1.68^{a-c}	2.30^{b-h}	15	55.69	1.65	4.12^{b-f}	7	60.9^{e-g}	1.9^{a-d}	5.96^{b-e}	6	62.8^{f-i}	0.8^{a-d}
G6	CIMMYTexp 3	4.77^{bc}	4	60.52^{f-i}	0.60^{f}	3.20^{ab}	2	57.39	0.83	4.38^{b-d}	5	61.7^{c-f}	0.7^{e}	6.13^{a-c}	4	61.8^{ij}	0^{e-f}
G7	CIMMYTexp 4	4.67^{b-d}	5	60.59^{e-i}	1.76^{ab}	2.91^{a-e}	5	54	2.31	4.40^{b-d}	4	61.5^{c-f}	1.8^{a-d}	5.96^{b-e}	7	63.6^{c-g}	0.8^{a-e}
G8	CIMMYTexp 5	5.47^{a}	1	62.41^{a-d}	1.51^{a-d}	2.88^{a-f}	6	57.83	1.16	5.27^{a}	1	63.4^{ab}	1.7^{a-d}	7.08^{a}	1	64.3^{b-e}	0.8^{a-e}
G9	AGR1	3.36^{j-l}	23	61.33^{c-g}	1.55^{a-d}	2.14^{c-h}	17	51.41	1.33	3.10^{ij}	22	62.9^{a-c}	1.9^{a-c}	4.36^{i-k}	22	64.8^{a-c}	0.8^{a-e}
G10	MKS1	3.46^{j-l}	21	61.52^{b-g}	1.16^{c-e}	2.56^{a-h}	11	59.15	1.32	3.13^{h-j}	21	62.0^{b-f}	1.2^{de}	4.40^{h-k}	20	63^{e-i}	0.3^{c-f}
G11	MKS2	3.65^{g-k}	18	58.16^{kl}	1.07^{d-f}	2.55^{a-h}	12	52.17	0.5	3.03^{ij}	23	59.2^{h}	1.5^{a-d}	5.13^{d-i}	15	60.2^{kl}	0.3^{c-f}
G12	PAN1	4.66^{b-d}	6	63.04^{ab}	1.81^{a}	2.02^{e-h}	22	60.53	2.48	4.59^{a-c}	3	62.9^{a-c}	1.6^{a-d}	6.10^{a-d}	5	65.3^{ab}	1.3^{a-b}
G13	PAN2	4.17^{d-g}	10	61.56^{b-g}	1.82^{a}	2.12^{c-h}	18	58.16	2.98	3.89^{c-h}	11	62.1^{b-f}	1.8^{a-d}	5.61^{c-f}	9	63.3^{d-h}	0.6^{b-f}
G14	PNR1	4.39^{c-e}	8	58.07^{kl}	1.27^{a-e}	2.43^{b-h}	13	52.15	1.51	4.07^{b-f}	8	59.0^{h}	1.4^{a-e}	5.84^{b-e}	8	60.3^{kl}	0.8^{a-e}
G15	PNR2	4.02^{e-i}	12	59.42^{h-k}	1.24^{b-e}	2.17^{c-h}	16	58.56	1.17	4^{c-f}	9	59.8^{gh}	1.3^{b-e}	4.98^{e-j}	16	60.3^{kl}	0.8^{a-e}
G16	SC1	4.94^{b}	2	61.21^{d-g}	1.33^{a-d}	3.02^{a-c}	3	56.11	1.48	4.76^{ab}	2	61.6^{c-f}	1.2^{c-e}	6.16^{a-c}	3	63.7^{c-f}	1^{a-c}
G17	SC2	3.26^{kl}	24	60.63^{e-h}	1.18^{c-e}	2.58^{a-h}	9	55.69	1.84	2.93^{j}	25	62.1^{b-f}	1.2^{c-e}	4.10^{jk}	24	62.1^{h-j}	0.4^{c-f}
G18	SC3	3.10^{l}	25	62.19^{a-e}	1.16^{c-e}	1.81^{gh}	24	54.1	1.81	3.01^{ij}	24	62.5^{a-e}	1.3^{b-e}	3.90^{k}	25	65.9^{a}	-0.1^{f}
G19	SC4	3.40^{j-l}	22	58.61^{jk}	1.70^{a-c}	2.08^{d-h}	19	53.97	1.65	3.20^{g-j}	20	58.3^{hi}	2^{ab}	4.37^{i-k}	21	61.3^{jk}	0.8^{a-d}
G20	SC5	3.99^{e-i}	13	60.2^{g-j}	1.21^{b-e}	2.66^{a-g}	7	55.82	1	3.50^{e-j}	15	60.8^{fg}	1.5^{a-d}	5.4^{c-g}	10	62.3^{g-j}	0.3^{c-f}

G21	SC6	3.76^{g-k}	17	56.92^{l}	1.16^{c-f}	2.03^{e-h}	20	52.43	0.5	3.88^{c-h}	12	57.1^{i}	1.5^{a-d}	4.45^{g-k}	19	59.2^{i}	0.7^{a-f}
G22	SYNG1	4.09^{e-h}	11	63.41^{a}	1.44^{a-d}	2.64^{a-h}	8	60.51	1.48	3.76^{d-i}	13	62.7^{ad}	1.6^{a-d}	5.31^{c-i}	12	65.5^{ab}	0.8^{a-e}
G23	SVAN1	3.82^{f-j}	15	62^{a-f}	1.53^{a-d}	2.42^{b-h}	14	51.65	1.38	3.67^{d-j}	14	63.4^{ab}	1.6^{a-d}	4.75^{f-k}	17	64.8^{a-c}	0.8^{a-d}
G24	BYR1	3.61^{h-l}	19	59.44^{h-k}	1.52^{a-d}	2.56^{a-h}	10	52.29	1.98	3.49^{e-j}	16	60.9^{fg}	1.7^{a-d}	4.33^{i-k}	23	61.8^{ij}	0.5^{c-f}
G25	BYR2	3.50^{i-l}	20	58.97^{i-k}	1.59^{a-d}	1.94^{f-h}	23	58.2	0.66	3.36^{f-j}	19	58.5^{hi}	1.7	4.50^{g-k}	18	60.3^{kl}	1.4^{a}
Mean		4.05		61.78	0.68	2.42		55.59	1.45	3.91		61.1	1.67	5.22		62.8	1.63
LSD		0.51		0.99	0.56	0.41		0	0	0.74		1.29	0	0.8		2.38	1.82
CV		32.9		4.63	77.28	37.76		12.37	126	40.78		4.74	64.1	24.99		4.49	148.23
PValue		***		***	**	**		ns	ns	***		***	ns	***		***	*
H^2		0.67		0.83	0.35	0.17		0	0	0.45		0.7	0	0.35		0.71	0

*, **, *** indicate mean squares significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ probability levels, respectively, ns = not significant, CV= coefficient of variation, LSD= least significant differences, G. mean = grand mean, H^2 = broad sense heritability

5.3.2 Identification of commercial hybrids specifically adapted under heat and combined drought and heat stress conditions

Figure 5.1 displays the "which-won-where" polygon-view results for GYD under each sector in the GGE biplot. The biplot explained 90.15% of the overall variation regarding G and GEI with PC1 and PC2 accounting for 63.41% and 26.75% of the variation, respectively. The vertex genotype has the longest vectors and represents the highest yielding genotype in the location that fell within that particular sector (Yan et al. 2001; Sibiya et al. 2012). Accordingly, the vertex genotypes (G12, G16, G4, G17, and G18) were among the most responsive (either best or worst) to environmental interactions for GYD in their corresponding directions in a given sector (Figure 5.1). Six sectors were generated by the GGE biplot's rays from the origin but the environments were present in only three of the sectors. The first sector had genotypes specifically adapted to heat stress and G4 was the highest yielding genotype which was at the vertex in this sector. The second and third sectors had genotypes specifically adapted to RDHS and OC and the vertex genotypes were G12 and G16 respectively (Figure 5.1).

5.3.3 Adaptability and stability of genotypes across environments

Figure 5.2 shows the mean yield performance and stability of genotypes evaluated over two years across six locations, under HTS, RDHS and OC conditions. The biplot explained 94.89% of the total variation observed in grain yield, of which 82.97% was explained by the first principal component (PC1), while the second principal component (PC2) explained 11.92%. In the average environmental coordinate (AEC) system, AEC X-axis (abscissa) passes through the biplot origin with an arrow that points to the axis' positive end and the genotypes' mean performance. The AEC Y-axis (ordinate) passes through the biplot origin and is perpendicular to the AEC X-axis (Yan et al. 2001). According to Figure 5.2, genotypes with above average yield were located on the right side of the biplot origin, while genotypes with below average yield were located on left side. The highest yielding genotype was G8 followed by G16 (4.94 t ha^{-1}), G3 (4.79 t ha^{-1}) and G6. The stability of the genotypes was measured by their projections onto the AEC ordinates. Genotype G6 and G7 were the most stable because of their short projection onto the AEC ordinate. The experimental genotype, G8 (5.47 t ha^{-1}), was overall, the highest performing genotype but was unstable (Figure 5.2; Table 5.5).

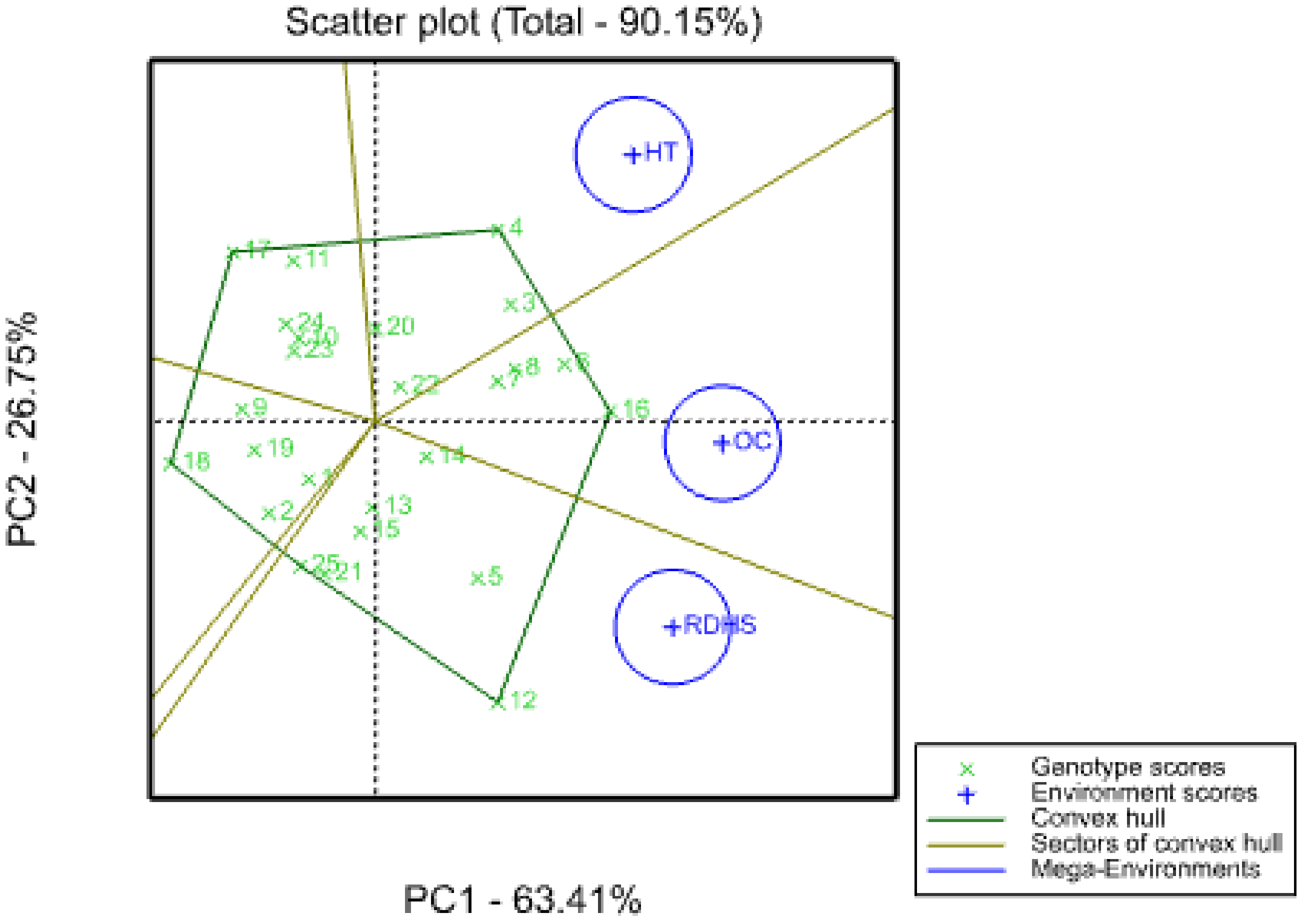


Figure 5.1 GGE biplot of the average environment coordination (AEC) view showing commercial and experimental hybrids adapted under HT, RDHS and OC in Zimbabwe (Yan and Tinker, 2006) HT = managed heat stress conditions, OC = optimal conditions, and RDHS = random drought and heat stress conditions.

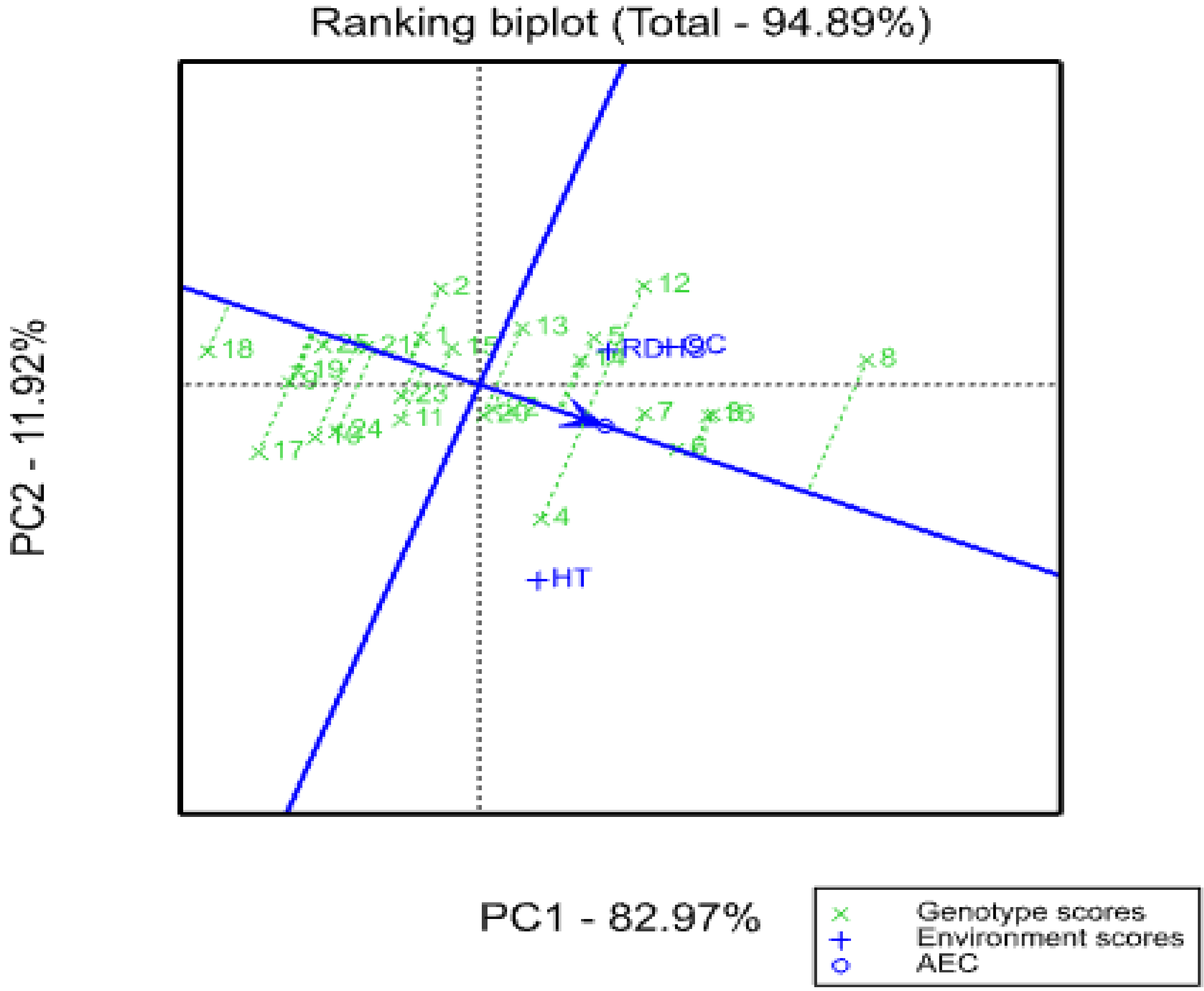


Figure 5.2 GGE biplot of the average environment coordination (AEC) view showing mean grain yield performance and stability of 25 maize hybrids (20 commercial + 5 checks) evaluated over two years across six locations, under three management regimes (HTS, RDHS and OC) in Zimbabwe (Yan and Tinker, 2006). HT = heat stress conditions, OC = optimal conditions, and RDHS = random drought and heat stress conditions**.**

5.4 Discussion

In any breeding programme, the initial and essential step in search of the required genetics and stabilizing yield is to evaluate the existing germplasm under the prevailing stress conditions (Meseka et al. 2018). Varietal evaluation provides important information for selecting and recommending a genotype (Matongera et al. 2023). Because of the significant GYD losses experienced in Zimbabwe due to heat stress, the current study evaluated the existing early maturing maize commercial hybrids that are currently on the market together with experimental hybrids selected for heat stress adaption in Zimbabwe. The study was hinged on the hypothesis that, among the early maturing maize hybrids already released for cultivation in Zimbabwe, hybrids adapted and productive under heat and combined drought and heat stress factors exist. Additionally, commercial hybrids adapted and productive under stress can be complemented on the market by maize genotypes specifically developed for heat stress tolerance to enhance maize productivity under stress conditions in SSA. Results of this study demonstrated the potential of the already commercialized maize hybrids in boosting yields under heat and combined drought and heat stress prone agro-ecologies in SSA. Furthermore, experimental maize hybrids developed for heat stress adaptation that can potentially complement the already existing commercial hybrids under heat as well as the combined drought and heat stress environments were identified.

To begin with, the significant differences observed among the hybrids for GYD, AD and ASI across environments point to the presence of genotypic differences among the hybrids evaluated. The differences could be attributed to the different genetic makeup and background of the material used in the formation of the hybrids used in the study (Bruce et al. 2002). The highly significant differences observed on GYD, AD and ASI demonstrated that there is adequate genetic variation for effective heat stress breeding in SSA. These results concur with the results of Pavani et al. (2019) who reported significant differences among the hybrids that were evaluated under contrasting management conditions for grain yield. Similar results were also reported by Abera et al. (2004) who observed differences among genotypes evaluated under heat stress and non-stress conditions. Pavani et al. (2019) observed significant differences on GYD and AD under heat stress conditions.

The significant ($P \leq 0.01$) interaction of hybrids and locations for GYD and ASI observed in this study suggests the influence of the environment on the expression of these traits. The results reflect that hybrids have different adaptive mechanisms in different environments (Bruce et al. 2002). These results concur with those of Thayil et al. (2020) who observed significant GEI under heat stress for GYD with some entries displaying significant crossover interaction across the years. The differential performance of the genotypes observed across environments complicates the selection of best genotypes across environments (Pavani et al. 2019; Abakemal and Shimelis, 2020). The significant ($P \leq 0.01$) season and location interaction for all the traits measured during the study, implies that the genetic potential of the hybrids was influenced by the year and location variation. Similar results were reported by Beyene et al. (2012) and Kolewole et al. (2021).

The non-significant GEI observed on AD implies that flowering of the genotypes was consistent across the environments. The results concur with those reported by Dojamo et al. (2022) under stress environments. Similarly, non-significant hybrid x year interaction observed on GYD and AD demonstrated consistent performance of genotypes over the years of evaluation, hence aiding in the identification of potentially high yielding and stable genotypes. These results are in agreement with the results of the research conducted by Kolewole et al. (2021). In the absence of significant interaction between the hybrids and years for GYD in maize, some researchers have identified superior hybrids (Abera et al. 2004; Kolewole et al. 2021).

The heritability of a trait is deemed very high if it is 80% or more, moderate if it falls between 40 and 80%, and low if it is less than 40% (Singh, 2001). High broad-sense heritability observed on AD across all the environments is an indication of the effect of some major genes and negligible influence of the environment on the trait (Gazala et al. 2019). These results are similar to the results by Gazala et al. (2019) who observed high broad sense heritability value for flowering. The high broad-sense heritability observed for GYD under heat stress, optimal, and across environments suggests that there is substantial genetic variation, and it can be effectively targeted for genetic improvement through selective breeding and advanced breeding techniques. Selection for improved GYD in the set of hybrids that were evaluated in this study would be effective because of the high heritability values for GYD that were observed under RDHS, OC, and across conditions.

The grain yield performance of the hybrids evaluated varied substantially across different management conditions. As anticipated, lower mean GYD was observed under HTS and RDHS conditions compared to the OC. Under different stress conditions, varying degrees of yield reduction could be anticipated based on the crop's level of exposure to stress (Derera et al. 2008; Mebratu et al. 2019). The benefit of a humid atmosphere over a drier one is demonstrated by the higher mean GYD that was observed under OC conditions as compared to HTS and RDHS conditions. This results in increased dry matter production and ultimately higher yields. These results are consistent with the results of Thayil et al. (2020) who reported higher mean yield under optimal compared to stress condition.

The superior performance exhibited by the early maturing commercial hybrids, G3 and G16, under heat stress is quite encouraging because these hybrids can be recommended for production under heat stress prone agro-ecologies in SSA while breeding for heat stress tolerance is ongoing. Although, the commercial hybrids evaluated were not bred for heat tolerance, the results demonstrated that they have some level of tolerance to heat stress as they performed better than some of the experimental hybrids specifically selected for heat stress adaptation. The performance of these two commercial varieties is also promising to plant breeders who are pursuing the development of high yielding heat tolerant hybrids, because parents of these respective hybrids can be screened under heat stress in order to depict possible source materials for heat stress breeding in SSA. Previous studies have identified heat stress tolerant maize hybrids from the evaluation of maize genotypes under HTS conditions (Koirala et al. 2017; Archana et al. 2018; Pavani et al. 2019).

The heat tolerant experimental hybrids, G4 and G6, which exhibited superior performance over all the hybrids in the study under heat stress condition, can be recommended for release and production in heat stress prone agro-ecologies. Superior performance of these experimental hybrids is not surprising since they were selected for heat stress tolerance. Yield gain of stress tolerant experimental hybrids over the commercial hybrids has been previously reported in southern Africa (Setimela et al. 2016) and eastern Africa (Njeri et al. 2017).

Drought and heat stress usually occur simultaneously in the farmer’s fields. It is interesting to note that, there are commercial hybrids G12 and G16 which performed very well under RDHS conditions hence, they can be recommended for production in heat and drought stress prone areas. The parents of these two commercial hybrids can be further evaluated under drought and heat stress conditions to identify tolerant lines that can be used as genetic resources for combined heat and drought stress breeding in maize. The experimental hybrids G7 and G8 that showed superior performance under RDHS proved that they are a good fit under RDHS, hence they can be recommended for release so that famers in heat and drought prone areas can make use of them.

The GGE biplot displays a polygon view of the “which won-where” pattern by segmenting the environments into distinct sectors, and each sector have a unique vertex genotype that reflects the most adaptable and highest yielding genotype (Yan and Tinker, 2006). The biplot also aids in visualizing the patterns of interaction between genotypes and environments, to show the existence of crossover GEI, and specific adaptation (Yan and Tinker, 2006). Farmers that are primarily interested in a cultivar's performance in their area would prefer specific adaptability (Kang, 1998). Considering the performance of genotype G4 under HTS condition the genotype could be recommended for specific deployment in heat stress environment where the genotype performed relatively well.

Regarding the ranking biplot across environments, the high yielding and stable commercial hybrids (G3 and G16) and the experimental hybrids (G6, G7), could be recommended for production across stress and non-stress agro-ecologies and their parents can be used for adaptability breeding. Stable genotypes across different environmental conditions are favored by breeders for seed production because they are less susceptible to environmental fluctuations. The adapted and stable hybrids identified will improve maize yields across maize growing regions of Zimbabwe and enhance sustainable productivity. Apart from this, genotypes with high GYD stability and adaptability improve farmers' income and contribute to national and household food security (Ceccareli, 1996).

5.5 Conclusion and Recommendations

This study has identified early maturing high yielding commercial hybrids G3 and G16, which performed well under heat stress condition as good candidates for immediate use under heat stress conditions. The two experimental hybrids G4 and G6 that showed superior performance and were selected for heat stress adaptation can be recommended for release and production in heat stress prone areas. It can be inferred in this study that high level of genetic variability was present among maize hybrids for GYD under HT (Koirala et al. 2017). The high yielding commercial hybrids identified under heat stress can be recommended for production in heat prone areas of Zimbabwe and their parents can be used as base germplasm for developing inbred lines with higher levels of tolerance to heat stress (Mukaro et al. 2023).

References

Abakemal, D.; Shimelis. H. Genotype-by-environment interaction and yield stability of quality protein maize hybrids developed from tropical- highland adapted inbred lines. *Euphytica* 2020. doi: 10.1007/s10681-016-1673-7.

Abera, W.; van Rensburg, J.B.J.; Labuschagne, M.T.; Maartens, H. Genotype-environment interactions and yield stability analyses of maize in Ethiopia. *South African J. Plant Soil* 2004.*21*(4), 251-254, doi: 10.1080/02571862.2004.10635058.

Akter, N.; Rafiqul, I. Heat stress effects and management in wheat. A review. Agron. Sustain. Dev. 2017, 37(5), 1-17. doi: 10.1007/s13593-017-0443-9.

Ali, F.; Kanwal, N.; Ahsan, M.; Ali, Q.; Bibi, I.; Niazi, N.K. Multivariate analysis of grain yield and its attributing traits in different maize hybrids grown under heat and drought stress. *Scientifica*, 2015. 1–6. doi: 10.1155/2015/563869.

Archana, K.A.; Kuchanur, P.H.; Zaidi, P.H.; Mandal, S.S.; Arunkumar, B.; Patil, A.; Vinayan, M.T.; Seetharam, K. Combining ability and heterosis in tropical maize (Zea mays L.) under heat stress. *J. pharmacogn. phytochem.* 2018, *7*(6), 1026-1031.

Badu-Apraku, B.; Hunter, R.B.; Tollenaar, M. Effect of temperature during grain filling on whole plant and grain yield in maize. (*Zea mays L.*). *Can. J. Plant Sci*. 1983, 63(2), 357–363. doi: 10.4141/cjps83-040.

Badu-Apraku, B.; Menkir, A.; Ajala, S.; Akinwale, R.; Oyekunle, M.; Obeng-Antwi, K. Performance of tropical early-maturing maize cultivars in multiple stress environments. *Can. J. Plant Sci.* 2010, *90*(6), 831-852. doi: 10.4141/CJPS10059.

Beyene, Y.; Mugo, S.; Tefera, T.; Gethi, J.; Gakunga, J.; Ajanga, S.; Karaya, H.; Musila, R.; Muasya, W.; Tende, R.; Njoka, S. Yield stability of stem borer resistant maize hybrids evaluated in regional trials in East Africa. *African J. Plant Sci*. 2012, 6(2), 77–83. doi: 10.5897/ajps11.262.

Bocianowski, J.; Nowosad, K.; and Tomkowiak, A. Genotype–environment interaction for seed yield of maize hybrids and lines using the AMMI model. *Maydica*, 2019, *64*(2), 1-8.

Brown, I.; Poggio, L.; Gimona, A.; Castellazzi, M.. Climate change, drought risk and land capability for agriculture: implications for land use in Scotland. *Reg Environ Change*. 2011, **11**, 503–518. https://doi.org/10.1007/s10113-010-0163-z

Bruce, W.B.; Edmeades, G.O.; Barker, T.C. Molecular and physiological approaches to maize improvement for drought tolerance. *J. Exp. Bot.* 2002. *53*(366), 13-25. doi: 10.1093/jxb/53.366.13.

Cairns, J.E.; Crossa, J.; Zaidi, P.H.; Grudloyma, P.; Sanchez, C.; Araus, J.L.; Thaitad, S.; Makumbi, D.; Magorokosho, C.; Bänziger, M.; Menkir, A. Identification of drought, heat, and combined drought and heat tolerant donors in maize. *Crop Sci*, 2013. *53*(4),1335-1346. doi: 10.2135/cropsci2012.09.0545.

Cairns, J.E.; Sonder, K.; Zaidi, P.H.; Verhulst, N.; Mahuku, G.; Babu, R.; Nair, S.K.; Das, B.; Govaerts, B.; Vinayan, M.T.; Rashid, Z. Maize production in a changing climate: impacts, adaptation, and mitigation strategies. *Adv. Agron.* 2012, *114*, 1-58: doi: 10.1016/B978-0-12-394275-3.00006-7.

Ceccarelli, S. Positive interpretation of genotype by environment interactions in relation to sustainability and biodiversity. In Cooper, M., Hammer, G.L. (Eds) *Plant adaptation and crop improvement. 1996.* CABI International, United Kingdom pp.467-486.

Couto, M.F.; Nascimento, M.; do Amaral Jr, A.T.; e Silva, F.F.; Viana, A.P.; Vivas, M. Eberhart and Russel's Bayesian Method in the selection of popcorn cultivars. *Crop Science*, 2015, *55*(2), 571-577. doi: 10.2135/cropsci2014.07.0498.

Derera, J.; Tongoona, P.; Vivek, B.S.; Laing, M.D. Gene action controlling grain yield and secondary traits in southern African maize hybrids under drought and non-drought environments. *Euphytica*, 2008. 162(3): 411–422. doi: 10.1007/s10681-007-9582-4.

Dojamo,T.S.; Markos Takiso, S.; Lema Tessema, M. Evaluation of Maize (Zea mays L.)Varieties in Selected Lowland Areas of Southern Ethiopia. *Int. J. Agron.* 2022, 9690792 https://doi.org/10.1155/2022/9690792

FAOSTAT. 2021. World Food and Agriculture – Statistical Yearbook 2021.

Fonseca, A.E.; Westgate, M.E. Relationship between desiccation and viability of maize pollen. *F. Crop. Res.* 2005.*94*(2-3), 114-125. doi: 10.1016/j.fcr.2004.12.001.

Gazala, P.; Kuchanur, P.H.; Zaidi, P.H.; Arunkumar, B.; Patil, A.; Seetharam, K.; Vinayan, M.T. 2019. Evaluation of tropical maize hybrids for seed yield and its related traits under heat stress environment (Zea mays L.). *J. Pharmacogn. Phytochem.* 2019, *8*(4), 1840-1845.

Hallauer, A.R.; Carena, M.J.; Miranda Filho, J.D. Quantitative genetics in maize breeding 2010, 3rd edn. Iowa State University Press, Ames

Hasanuzzaman, M.; Nahar, K.; Alam, M.M.; Roychowdhury, R.; Fujita, M. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *Int. J. Mol. Sci*. 2013, *14*(5), 9643-9684: https://doi.org/10.3390/ijms14059643

Intergovernmental Panel on Climate change. 2016. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth ASSESSMENT Report.

Kang, M.S. Using genotype-by environment interaction for crop cultivar development. Adv. Agron. 1998. 62, 199–252. https://doi.org/10.1016/S0065-2113(08) 60569-6.

Khodarahmpour, Z.; Choukan, R.; Bihamta, M.R.; Majidi, H.E. Determination of the best heat stress tolerance indices in maize (*Zea mays L.*) inbred lines and hybrids under Khuzestan province conditions. *J. Agric. Sci. Technol*. 2011. 13(1), 111–121.

Koirala, K.B.; Giri, Y.P.; Rijal, T.R.; Zaidi, P.H.; Ajanahalli, R.S.; Shrestha, J. Evaluation of grain yield of heat stress resilient maize hybrids in Nepal. *Int. J. Appl. Sci. Biotechnol*. 2017, 5(4): 511–522. doi: 10.3126/ijasbt.v5i4.18774.

Koirala, K.B.; Tripathi, M.P.; Seetharam, K.; Vinayan, M.T.; and Zaidi, P.H. Field evaluation of heat stress-resilient maize hybrids for improved and stable maize production in Nepal. *SAARC J. Agric*. 2021, 19(1), 27–43. doi: 10.3329/sja.v19i1.54776.

Kolawole, A.O.; Raji, I.A.; Oyekale, S.A. The performance of new early maturing pro-vitamin A maize (Zea mays L.) hybrids in the derived savanna agro-ecology of Nigeria. *J. Agric. Sci.* 2021.*66*(3), 231-245. https://doi.org/10.2298/JAS2103231K

Lamaoui, M.; Jemo, M.; Datla, R.; Bekkaoui, F. Heat and drought stresses in crops and approaches for their mitigation. *Front. Chem.* 2018, 6, 26: https://doi.org/10.3389/fchem.2018.00026

Lobell, D.B.; Bänziger, M.; Magorokosho, C; Vivek, B. Nonlinear heat effects on African maize as evidenced by historical yield trials. Nat. Clim.Chang, 2011, 1(1), 42–45, doi: 10.1038/nclimate1043.

Lobell, D.B.; Sibley, A.; Ivan Ortiz-Monasterio, J. Extreme heat effects on wheat senescence in India. *Nat. Clim. Chang.* 2012.*2*(3), 186-189: doi: 10.1038/nclimate1356.

Magorokosho, C.; Vivek, B.; MacRobert, J.F.; Tarekegne, A.T. Characterization of maize germplasm grown in eastern and southern Africa: Results of the 2009 regional trials coordinated by CIMMYT. 2010. CIMMYT, Harare, Zimbabwe.

Malato, G. An Introduction to the Shapiro-Wilk Test for Normality. 2023.

Matongera, N.; Ndhlela, T.; van Biljon, A.; Labuschagne, M. Genotype x environment interaction and yield stability of normal and biofortified maize inbred lines in stress and non-stress environments. *Cogent Food Agric.* 2023.*9*(1). 9:1, DOI: 10.1080/23311932.2022.2163868

Matova, P.M.; Kamutando, C.N.; Mutari, B.; Magorokosho, C.; Labuschagne, M. Adaptability and Stability Analysis of Commercial Cultivars, Experimental Hybrids and Lines under Natural Fall Armyworm Infestation in Zimbabwe Using Different Stability Models. *Int. J. Agron.* 2022. *12*(7), 1724. doi: 10.3390/agronomy12071724.

Mebratu, A.; Wegary, D.; Mohammed, W.; Teklewold, A.; and Tarekegne, A. Genotype× environment interaction of quality protein maize hybrids under contrasting management conditions in Eastern and Southern Africa. *Crop Sci*, 2019.*59*(4), 1576-1589. doi: 10.2135/cropsci2018.12.0722.

Meseka, S.; Menkir, A.; Bossey, B.; Mengesha, W. Performance assessment of drought tolerant maize hybrids under combined drought and heat stress. *J. Agron.* 2018.*8*(12), 274: doi: 10.3390/agronomy8120274.

Monsanto. Heat and drought stress impactson corn: Technology development and Agronomy. Agron. Alert 2012.

Mukaro, R.; Kamutando, C.N.; Magorokosho, C.; Mutari, B.; Zaidi, P.H.; Kutywayo, D.; Sibiya, J. Genetic potential of tropically adapted exotic maize (Zea mays L.) heat-tolerant donor lines in sub-tropical breeding programs. *J. Agron*, 2023. *13*(8), 1-6: https://doi.org/10.3390/agronomy13082050

Mutari, B.; Sibiya, J.; Bogweh Nchanji, E.; Simango, K.; Gasura, E. Farmers' perceptions of navy bean (*Phaseolus vulgaris L.*) production constraints, preferred traits and farming systems and their implications on bean breeding: a case study from South East Lowveld region of Zimbabwe. *J. Ethnobiol. Ethnomed.* 2021, 17(1): 1–20. doi: 10.1186/s13002-021-00442-3.

Naveed, M.; Ahsan, M.; Akram, H.M.; Aslam, M.; and Ahmed, N. Genetic effects conferring heat tolerance in a cross of tolerant× susceptible maize (Zea mays L.) genotypes. *Front. Plant Sci.* 2016, *7*, 1-12: doi: 10.3389/fpls.2016.00729.

Nelimor, C.; Badu-Apraku, B.; Tetteh, A.Y.; N'guetta, A.S. Assessment of genetic diversity for drought, heat and combined drought and heat stress tolerance in early maturing maize landraces. *Plants*. 2019.*8*(11), 1-9: doi: 10.3390/plants8110518.

Njeri, S.G.; Makumbi, D.; Warburton, M.L.; Diallo, A.; Jumbo, M.B.; Chemining'wa, G. Genetic analysis of tropical quality protein maize (Zea mays L.) germplasm. *Euphytica*, 2017, 213(11): 1–19. doi: 10.1007/s10681-017-2048-4.

Nyaga, C.; Gowda, M.; Beyene, Y.; Muriithi, W.T.; Makumbi, D.; Olsen, M.S.; Suresh, L.M.; Bright, J.M.; Das, B.; Prasanna, B.M. Genome-wide analyses and prediction of resistance to MLN in large tropical maize germplasm. *Genet. genomics.* 2019, *11*(1), 1–16. doi: https://doi.org/10.3390/genes11010016.

Oliveira, E.J.D.; Freitas, J.P.X.D.; Jesus, O.N.D. AMMI analysis of the adaptability and yield stability of yellow passion fruit varieties. *Sci. Agric.*, 2014. 71(2): 139–145. doi: 10.1590/S0103-90162014000200008.

Patterson, H.D.; Williams, E. A new class of resolvable incomplete block designs. *Biometrika*, 1976, 63(1), 83–92. doi: https://doi.org/10.1093/biomet/63.1.83.

Pavani, N.; Kuchanur, P.H.; Patil, A.; Arunkumar, B.; Zaidi, P.H.; Vinayan, M.T.; Seetharam, K. Stability analysis of stress-resilient maize (Zea mays L.) hybrids across stressed and non-stressed environments. Int.J.Curr.Microbiol.App.Sci. 2019, (9): 252-260. doi: http://www.ijcmas.com/.

Payne, R.W., Murray, D.A., Harding, S.A., 2018. An Introduction to GenStat Command Language. VSN International, Hampstead, UK.

Prasanna, B.M.; Cairns, J.E.; Zaidi, P.H.; Beyene, Y.; Makumbi, D.; Gowda, M.; Magorokosho, C.; Zaman-Allah, M.; Olsen, M.; Das, A.; Worku, M.; Beat the stress: breeding for climate resilience in maize for the tropical rainfed environments. *Theor. Appl. Genet. 134*(6). 2021,1729-1752: doi: 10.1007/s00122-021-03773-7.

Rahman, S.U.; Arif, M.; Hussain, K.; Arshad, M.; Hussain, S.; Mukhtar, T.; Razaq, A. Breeding for heat stress tolerance of maize in Pakistan. *J. Environ. Agric. Sci*, 2015.*5,* 27-33.

Rahman, S.U.; Arif, M.; Hussain, K.; Hussain, S.; Mukhtar, T.; Razaq, A.; Iqbal, R.A.; 2013. Evaluation of maize hybrids for tolerance to high temperature stress in central Punjab. *Am. J. Bioeng. Biotechnol.2013, 1*(1), 30-36. doi: 10.7726/ajbebt.2013.1003.

Rezende, W.S.; Beyene, Y.; Mugo, S.; Ndou, E.; Gowda, M.; Sserumaga, J.P.; Asea, G.; Ngolinda, I.; Jumbo, M.; Oikeh, S.O.; Olsen, M. Performance and yield stability of maize hybrids in stress-prone environments in eastern Africa. *Crop J.* 2020. *8*(1), 107-118. doi: 10.1016/j.cj.2019.08.001.

Rosegrant, M.W.; Ringler, C.; Zhu, T. Water for agriculture: Maintaining food security under growing scarcity. *Annu. Rev. Environ. Resour.* 2009, *34: 205–222. doi: 10.1146/annurev.environ.030308.090351.*

Setimela, P.S.; Kassie, G.T.; Erenstein, O.; MacRobert, J.F.; Magorokosho, C.; Tarekegne, A.T.; Makumbi, D.; and Ndoro, O. Performance of elite drought tolerant maize varieties tested on farm in Eastern and Southern Africa.Season 15-16, 2016. http://hdl.handle.net/10883/19184

Sibiya, J.; Tongoona, P.; Derera, J; van Rij, N. Genetic analysis and genotype× environment (G× E) for grey leaf spot disease resistance in elite African maize (Zea mays L.) germplasm. Euphytica, 2012. 185, 349-362: doi: 10.1007/s10681-011-0466-2.

Singh, S.P. Broadening the genetic base of common bean cultivars: A review. *Crop science*, 2001, *41*(6),1659-1675. https://doi.org/10.2135/cropsci2001.1659

Steel, R.G.; Torrie, J.H. Principles and procedures of statistics: A biometrical approach. *Am. Stat. Assoc.* 2014, 76(375): 753–754. http://www.jstor.org/stable/2287561 .

Tesfaye, K.; Kruseman, G.; Cairns, J.E.; Zaman-Allah, M.; Wegary, D.; Zaidi, P.H.; Boote, K.J.; Erenstein, O. Potential benefits of drought and heat tolerance for adapting maize to climate change in tropical environments. *Clim. Risk Manag.* 2018, *19*, 106-119: doi: 10.1016/j.crm.2017.10.001.

Thayil, V.M.; Zaidi, P.H.; Seetharam, K.; Rani Das, R.; Viswanadh, S.; Ahmed, S.; Miah, M.A.; Koirala, K.B.; Tripathi, M.P.; Arshad, M.; Pandey, K. Genotype-by-environment interaction effects under heat stress in tropical maize. *J. Agron.* 2020, 10(12), 1998: doi: 10.3390/agronomy10121998.

Thompson, L.M. Weather variability and the need for a food reserve. 1966. https://dr.lib.iastate.edu/handle/20.500.12876/12208.

Tiwari, Y.K.; Yadav, S.K. High temperature stress tolerance in maize (Zea mays L.): Physiological and molecular mechanisms. *J. Plant Biol.*, 2019, *62 (2),* 93-102: doi: 10.1007/s12374-018-0350-x.

Workie, A.; Zeleke, H.; Dessalegn, Y. Genotype X environment interaction of maize (Zea mays L.) across North Western Ethiopia. J. Plant Breed. Crop Sci. 2013. 5(9), 171–181. doi: 10.5897/jpbcs2013.0406.

Yan, W. GGE biplot: A windows application for graphical analysis of multi-environment trial data and other types of two-way data. Agron J. 2001, 93:1111–1118. https://doi.org/10.2134/agronj2001.9351111x

Yan, W. Estimation of the optimal number of replicates in crop variety trials. *Front. Plant Sci.*

2021.11, 590762 https://doi.org/10.3389/fpls.2020.590762.

Yan, W.; Tinker, N.A. Biplot analysis of multi-environment trial data: Principles and applications. Can. J. Plant Sci. 2006, 86(3), 623–645. doi: 10.4141/P05-169.

Yousaf, M.I.; Riaz, M.W.; Jiang, Y.; Yasir, M.; Aslam, M.Z.; Hussain, S.; Sajid Shah, S.A.; Shehzad, A.; Riasat, G.; Manzoor, M.A.; Akhtar, I. Concurrent effects of drought and heat stresses on physio-chemical attributes, antioxidant status and kernel quality traits in maize (Zea mays L.) hybrids. *Front. Plant Sci.* 2022. *13*, 1-14. doi.org/10.3389/fpls.2022.898823

Zaidi, P.H.; Zaman-Allah, M.; Trachsel, S.; Seetharam, K.; Cairns, J.E.; Vinayan, M.T. Phenotyping for abiotic stress tolerance in maize heat stress: A field manual. 2016. http://hdl.handle.net/10883/4821

CHAPTER 6

General discussion and conclusion

6.1 Introduction

This study aimed at bridging some of the gaps that exist in maize breeding for heat stress tolerance in Zimbabwe to help breeders in designing breeding strategy to improve grain yields under heat stress conditions. Significant yield losses recorded in SSA and Zimbabwe due to heat stress has affected the livelihoods and income of famers and at the same time the region has poor adaptive capacity to climate change. Genetic resistance has been reported a sustainable approach, hence the need to develop and identify high yielding heat stress tolerant maize varieties to use under stress conditions. The current study therefore, seeks to investigate the potential of breeding maize for heat tolerance in SSA. This chapter summarizes the key objectives, highlighting the major findings and their implication in breeding for heat stress tolerance.

6.2 Genetic Potential of Tropically Adapted Exotic Maize (*Zea mays* L.) Heat-Tolerant Donor Lines in Sub-Tropical Breeding Programmes

6.2.1 Major findings

Using the North Carolina Design II mating scheme, 15 locally adapted CIMMYT-Zimbabwe elite lines and 14 heat stress tolerant-donor lines from CIMMYT-India were crossed to ascertain the breeding value of each line. The design II analysis revealed significant GCA and SCA effects for exotic heat donor lines on grain yield under heat stress, optimal conditions, and across locations signifying the importance of additive and non-additive genetic inheritance for grain yield. Under heat stress, two single crosses (DJ265-15 × VL1018816 and DJ267-9 × CAL1440) showed positive significant SCA effects for grain yield, whereas three exotic HSTDLs (CAL14138, CAL152, and CAL1440) showed significant positive GCA effects. The exotic heat tolerant donor lines, (CAL14138, CAL152, and VL109126) demonstrated superior *per se* performance under heat, optimal and across environments.

6.2.2 Implications of the findings

The findings of this study suggest that sub-tropical breeding programmes could benefit from the use of heat tolerant donor lines to enhance maize's ability to withstand heat stress. The donor lines (CAL14138, CAL152, and CAL1440) that demonstrated strong combining ability for heat-stressed grain yield could be useful genetic resources for the introduction of heat-tolerant alleles into tropical breeding populations, thereby increasing genetic diversity and grain yield in native material. Single crosses (DJ265-15 × VL1018816 and DJ267-9 × CAL1440), that showed positive and significant SCA effects for grain yield under heat stress conditions could be used for extraction of desirable populations/ lines that combine heat tolerance and high grain yield potential and could be exploited for future breeding efforts.

6.3 Assessment of genetic diversity, relatedness and genetic purity of CIMMYT-India exotic heat tolerant maize donor lines and CIMMYT-Zimbabwe elite lines using SNP markers

6.3.1 Major findings of the study

In this research, the genetic purity, relatedness, and diversity of 35 inbred lines (14 heat tolerant donor lines from CIMMYT- India and 21 CIMMYT-Zimbabwe) were assessed using 94 single-nucleotide polymorphism (SNP) markers. The results showed that there is genetic diversity among the inbred lines that were evaluated. The genetic distance among the inbred lines ranged from 0.04 to 0.64. Inbred lines that were closely related includes CAL152 and CAL14138 (0.04), and CAL14138 and VL109126 (0.04). The inbred line pairs CAL14135 and ZL132077, CZL1112c and CZL16018, and CZL15153 and CZL16018 showed high level of dissimilar with genetic distance of 0.64 each.

The cluster analysis revealed 3 major clusters with random allocation of inbred lines. The first cluster was the largest with 24 (70.6%) inbred lines from both CIMMYT-Zimbabwe and CIMMYT-India sources. The second cluster comprises of 5 (14.7%) inbred lines from CIMMYT-Zimbabwe that are closely related in their pedigree information and heterotic grouping. The third cluster consisted of 5 (14.7%) inbred lines in which four lines were from CIMMYT- India and one from CIMMYT-Zimbabwe and all the lines in this cluster were in the same heterotic group.

Genetic purity analysis revealed that 30 out of 35 (85.78%) inbred lines evaluated were genetically pure with 54.28% being 100% pure. 13 out of 14 heat stress tolerant donor lines were genetically pure. 5 inbred lines failed genetic purity test with more than 5% heterozygosity.

6.3.2 Implication of the findings

The results obtained from this study will enable efficient and effective utilization of the genotyped inbred lines in the development of heat tolerant inbred lines and hybrids and to design a strong heat tolerant maize breeding programme in Zimbabwe and SSA. Closely related inbred lines identified can be used for breeding starts and generating heat tolerant breeding populations. Those that are not closely related can be used in hybrid breeding to develop divergent crosses for maximum heterosis.

6.4 Grain yield stability of early maturity commercial maize hybrids under heat stress prone agro-ecologies of Zimbabwe

6.4.1 Major findings

In this study early maturing commercial hybrids and heat tolerant experimental hybrids were evaluated under heat stress, random heat and drought as well as optimal conditions. The analysis of variance showed highly significant differences for grain yield an indication of sufficient genetic variability for effective heat stress breeding. The GGE ranking biplot identified genotype G3 and G16 as the best early maturing commercial hybrids under heat stress while G4 and G6 were identified as the outstanding heat stress experimental hybrids. The which-won-where ranking biplot identified G4 and G3 as the best hybrids under heat stress and random drought and heat stress conditions respectively. The stability analysis showed that G3 and G16 were high yielding and stable across environments while G8 was high yielding but unstable.

6.4.2 Implications of the findings

The results of this study imply that genetic diversity for heat stress tolerance is available among maize genotypes in the tropical breeding programmes. Hence famers should make use of the existing hybrids under heat stress to avert the problem of heat stress. The parents of the commercial hybrids that showed superior performance under heat stress (G3 and G16) can be screened under heat stress to identify those that can confer heat stress tolerance and recommended them for potential use in breeding for heat stress tolerance. Additionally, they can be used in hybrid combinations to develop high yielding heat stress tolerant hybrids and synthetics. The productive experimental genotypes selected for heat stress adaptation (G4 and G6), can be helpful in complementing the adapted commercial varieties under the defined stress prone agro-ecologies.

6.5 Conclusion and Future Perspective

The rising demand for maize use and population growth require continuous scientific efforts toward heat stress breeding in maize to improve grain yields. Breeding programmes in SSA need to invest in contemporary breeding technologies to be able to react quickly to heat stress challenges. Rapid technologies for pure line development such as doubled haploid, must be incorporated in heat stress tolerance breeding programmes to maximize heterosis and speed up the development of resistant hybrids. The availability of molecular markers could rather be put to use by forward and background selection for introgression of desirable genomic regions associated with heat tolerance. The use of marker assisted selection (MAS) is expected to improve the effectiveness of selection and trait introgression.

Maize has not been well researched in terms of the genetic and molecular basis of heat stress tolerance. Therefore, more research needs to be conducted to understand the mechanisms behind maize’s capability to tolerate heat stress. Having the knowledge of the mechanism will pave the way for the development of maize genotypes that can sustainably deliver economic yields under heat stress.

Integrating the insights from these three complementary studies, the maize breeding program can leverage the genetic resources of exotic heat-tolerant donor lines and local lines to develop superior, heat-tolerant hybrid varieties. The understanding of combining ability, genetic diversity, and commercial hybrid performance under heat stress will guide the strategic selection of parental lines, hybrid combinations, and ultimately, the release of novel, climate-resilient maize hybrids for the target production environments.