Genetic Analysis and Hybrid Prediction in Tropical Maize (*Zea mays* L.) Using Phenotypic and Single Nucleotide Polymorphic Markers

By

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THESIS SUMMARY

Maize (*Zea mays* L., 2n = 2x = 20) is a commodity crop serving the food, feed, and processing industries globally. The productivity of maize in Africa remains low (< 2 t/ha) due to various yield-limiting factors, including abiotic stresses (such as drought, heat stress, flooding, waterlogging, erosion and poor soil health), and biotic stresses (e.g. foliar diseases and insect pests). Limited adoption of new high yielding varieties, slow rate of varietal turnover , socio-economic constraints, and policy issues further hinder productivity. Seed Co Limited is a Pan-African seed company involved in the research, development, and commercialization of seeds of major food security grain crops, including maize. The Seed Co breeding program aims to enhance the yields of new generation maize cultivars via hybrid breeding by utilizing complementary and contrasting inbred lines. New lines and experimental hybrids are developed and phenotyped using economic agronomic traits and genotyped using high-resolution Single Nucleotide Polymorphism (SNP) markers to facilitate effective selection. Integrating phenotypic and genomic selection accelerates the development of inbred lines with desirable traits to create high-performing single crosses and three-way hybrids. The new hybrids should undergo rigorous field testing for yield gains and stability across various locations to guide cultivar release and commercialization. Therefore, to complement this breeding initiative, the objectives of the study were:

i. to assess a maize germplasm panel's genetic diversity and population structure comprising 182 founder lines and 866 derived inbred lines using Single Nucleotide Polymorphism (SNP) markers to identify genetically unique lines for hybrid breeding,

ii. to conduct genome-wide prediction of yield and component traits using qualitative and quantitative phenotypic traits and SNP markers based on the additive-dominant genomic best linear unbiased predictions model to compute genomic estimated breeding values and genomic estimated genetic values to guide inbred line development and hybrid breeding,

iii. to assess the gains in yield and yield components among single cross maize hybrids selected through genomic prediction across representative locations to guide breeding and production, and

iv. to determine the combining ability effects of newly selected inbred lines and quantify the magnitude of heterosis and genotype by environmental interaction (GEI) effects of single cross hybrids to select and recommend contrasting elite lines and experimental hybrids.

In the first study, 182 founder and 866 derived maize inbred lines were characterized for genetic diversity and population structure analyses using SNP markers to identify genetically unique lines for hybrid breeding through beneficial allelic combinations. Genotyping was performed using the

Affymetrix platform for the 182 founder lines (1201 SNP markers) and the Midseq platform for the 866 derived lines (1484 markers). Moderate genetic variation with genetic distance ranging from 0.004 to 0.44 (mean: 0.25) for founder lines and 0.004 to 0.34 (mean: 0.13) for derived lines was observed. Heterozygosity values ranged from 0.00 to 0.24 for both lines. About 82% of the 1201 markers and 84% of the 1484 markers exhibited polymorphism information content ranging from 0.25 to 0.50, detecting a high level of genetic diversity and that the SNPs were highly informative in distinguishing the tested lines. Analysis of molecular variance revealed significant genetic differences ($P \leq 0.001$) among and within populations in the founder and derived lines. Notably, within-population variations accounted for 97% (founder lines) and 88.38% (derived lines) of the detected variations. Population structure analysis identified three subpopulations among founder lines and two among derived lines, which was supported by cluster analysis. Based on pairwise comparisons, genetically distant lines were selected, including G15NL337 and G15NL312 (Cluster 1), 15ARG152 and RGS-PL44 (Cluster 2), RGS-PL44 and 15ARG149 (Cluster 2), and RGS-PL33 and RGS-PL44 (Cluster 2). The selected lines are genetically distinct and recommended for marker-assisted hybrid maize breeding to leverage beneficial alleles.

The second study genotyped 1,102 genetically diverse inbred lines from two heterotic groups (N3 and SC) using high-density SNP markers. The 1,102 lines and 4 testers were crossed in a line-by-tester design to generate 2,830 single cross hybrids (SCHs). Phenotypic data were collected from field trials with the following SCHs: 684 evaluated at five locations in 2018/19, 760 at four locations (2019/20), 646 at four locations (2020/21), and 740 at four locations (2021/22) summer seasons in Zimbabwe. The trials were laid out in a 6 x 7 alpha lattice design with two replications at each site. 20 high-performing and contrasting inbred lines with the highest genomic estimated breeding values (GEBVs) and genomic estimated genetic values (GEGVs), each from the two heterotic groups, were identified for genetic advancement, combining ability tests and commercial hybrid development. 20 high-performing candidate SCHs with high GEGVs were identified for three-way hybrid development, variety registration and commercialization.

In the third study, 30 SCHs were developed from 11 inbred lines (6 from the N3 group and 5 from the SC group) with the highest predicted GEGVs for grain yield and associated traits using the genotypic best linear unbiased prediction (GBLUP) model. The lines were crossed using a factorial mating design with the six N3 lines used as female and five SC lines as male. The derived 30 SCHs and six commercial single cross check hybrids were field evaluated in seven locations, four in Zimbabwe and three in Zambia using a 6 x 6 alpha lattice design with two replications at each location. A combined analysis of variance revealed significant (P≤0.05) variation among the hybrids for the assessed 11 quantitative traits. Significant yield gains were realized over the mean of checks (at 13.09%), mean of the population (10.83%) and mean of best check (1.47%). Moderate to high broad-sense heritability (50 to 94%) and genetic advance were recorded for most of the assessed traits, indicating the success of selection assisted by genomic predictions. The study identified three best single cross hybrids (i.e., CTL03 x G16NL721, CTL03 x G17NL544 and GS-PL07 x G17NL544) with high and stable yields and recommended for commercialization.

In the fourth study, 11 elite inbred lines (6 female parents from N3 and 5 male parents from SC group) were crossed using a factorial mating design, resulting in 30 SCHs. The lines were selected based on the highest GEGVs for yield and component traits through GS using the GBLUP model. The 30 SCHs and six commercial check hybrids were field evaluated at seven locations (four in Zimbabwe and three in Zambia) during the 2022/2023 summer season. The trials were arranged in a 6 x 6 alpha lattice design with two replications at each location. Data were recorded on yield and yield components, and general combining ability (GCA) and specific combining ability (SCA) effects were computed. Significant GCA effects for grain yield (GY) were noted for lines CTL03, G17NL544, G16NL721, and GS-PL07, while significant SCA effects were recorded for crosses 15AG163 x G16NL679, G15NL304 x G17NL642, and 15AG162 x G16NL679. The additive main effects and multiplicative interaction (AMMI) model explained 38.95%, 50.58% and 7.24% of the total variation in GY due to genotype (G), environment (E), and genotype x environment interaction (GEI) effects in that order. The test locations were clustered into two mega environments: Rattray Arnold Research Station (RARS), Agricultural Research Trust (ART), Mpongwe Research Station (MPRS), and Lusaka West Research Station (LWRS) (Environment 1), and Mkushi Research Station (MKRS), Stapleford Research Centre (STAP), and Kadoma Research Centre (KRC) (Environment 2). The genotype and genotype-by-environment interaction (GGE) biplot analysis identified hybrids G15NL304 x G17NL544 and 15AG162 x G17NL544 as high-yielding and stable, suitable for commercialization. The two mega-environments and the selected stable, high-yielding general and specific combiners are recommended for genotype evaluation and production in Zimbabwe, Zambia, and comparable agroecologies.

Overall, the present study identified contrasting and genetically delineated inbred lines and enhanced the existing heterotic groups using high-throughput SNP markers. Best-performing lines (e.g. CTL03 and GS-PL07) were selected from the N3 heterotic group and G17NL544 and G16NL721 from the SC heterotic group. New single cross hybrids, such as CTL03 x G16NL721, CTL03 x G17NL544, and

GS-PL07 x G17NL544, were selected with grain yields of 8.38 t/ha, 8.24 t/ha, and 8.23 t/ha, respectively. The new experimental hybrids are recommended for three-way hybrid development or release following multi-environment evaluation.

DECLARATION

I, Rodreck Gunundu, declare that,

1. The research reported in this PhD thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any PhD degree or examination at any university.
3. This thesis contains no data, pictures, graphs, or other material from other people unless they are explicitly acknowledged as coming from other people.
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:
5. Their words have been rewritten or rephrased, but the general information attributed to them has been referenced.
6. Where exact words have been used, then their writing has been placed in italics and inside quotation marks and referenced.
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Signed

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RODRECK GUNUNDU

As the candidate supervisor, I agree to the submission of this thesis.

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Professor Hussein Shimelis (Supervisor)

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DEDICATION

This PhD thesis is lovingly dedicated to my beloved family - my wife, son, daughter, and parents - who have been my constant source of inspiration and strength. Their unwavering faith in me and their unrelenting optimism have been a beacon of hope, encouraging me to pursue my dreams, no matter how daunting they may seem. To them, and to all those who dare to dream big, I offer my deepest gratitude and dedication.

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ABBREVIATIONS AND ACRONYMS

AD	Days to anthesis
AEC	Average environment coordination
AFLP	Amplified fragment length polymorphism
AMMI	Additive main effects and multiplicative interaction model
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
ART	Agricultural Research Trust
ASI	Anthesis to silking interval
Bayes LASSO	Bayesian least absolute shrinkage and selector operator
BGLR	Bayesian generalized linear regression
BMS	Breeding Management System
BRNN	Bayesian regularized neural networks
BRR	Bayesian ridge regression
CHAK	Chakari
CIMMYT	International Maize and Wheat Improvement Center
CRISPR	Clustered regularly interspaced short palindromic repeats
CSI	Cultivar superiority index
CV	Coefficient of variation
DArTs	Diversity Array Technology
DF	Degrees of freedom
DH	Doubled haploid
DNA	Deoxyribonucleic acid
EH	Ear height
EHP	Number of ears harvested per $6m^2$
EPP	Number of ears per plant
ESA	Eastern and Southern Africa
GA	Genetic advance
GAM	Genetic advance as percentage of the mean
GBLUP	Genomic best linear unbiased prediction model
GCA	General combining ability

GCV	Genotypic coefficient of variation
GD	Genetic distance
GEBV	Genomic estimated breeding value
GEGV	Genomic estimated genetic value
GEI	Genotype x Environment Interaction
GGE	Genotype and Genotype x Environment
GLS	Gray leaf spot
G Matrix	Genomic relationship matrix
GS	Genomic selection
GWEN	Gwenhoro
GY	Grain yield
HC	Husk cover
HCno	Husk cover number
Het	Heterozygosity
HPH	High parent heterosis
IPCA	Interaction principal component axis
KASP	Kompetitive Allele Specific PCR
KRC	Kadoma Research Centre
LAI	Leaf area index
LASSO	Least absolute shrinkage selector operator
LD	Linkage disequilibrium
LSD	Least significant difference
LSR	Least square regression
LWRS	Lusaka West Research Station
MaF	Major allele frequency
MAF	Minor allele frequency
MAS	Marker assisted selection
Masl	Metres above sea level
MBC	Mean of best check hybrid
MC	Mean of commercial checks
ME	Mega environment
Micro-CT	Micro computed tomography

MKRS	Mkushi Research Station
MLR	Multiple linear regression
MOI	Moisture content of the grain
MP	Mean of population
MPRS	Mpongwe Research Station
MS	Mean of selected hybrids
MSE	Mean squares of error
MSG	Mean squares of genotypes
MSV	Maize streak virus
NP	Number of plants per $6m^2$
NJ	Neighbor joining
PCA	Principal component analysis
PCR	Polymerase chain reaction
PCV	Phenotypic coefficient of variation
PH	Plant height
PIC	Polymorphic information content
PLS	*Phaesopharia* leaf spot
QTL	Quantitative trait loci
RAPDS	Randomly Amplified Polymorphic DNAs
RARS	Rattray Arnold Research Station
rAmpSeq	Repeat Amplification Sequencing
RBFNN	Radial basis function neural networks
RF	Random forest
RFLP	Restriction fragment length polymorphism
RG	Realized gains
RGB camera	Red, green and blue wavelength camera
RKHS	Reproducing Kernel Hilbert Space
RL	Root lodging percentage
RR	Ridge regression
RR-BLUP	Ridge regression best linear unbiased prediction
RST	Rust
SCA	Specific combining ability

SCH	Single cross hybrid
SD	Days to silking/ Standard deviation
SE	Standard error
SL	Stalk lodging percentage
SNP	Single nucleotide polymorphism
SRC	Stapleford Research Centre
SS	Sum of squares
SSA	Sub Saharan Africa
SSR	Simple sequence repeats
ST	Sub-tropical
SVD	Single value decomposition
SVM	Support vector machine
TL	Total lodging percentage
TLS	Terrestrial laser scanning
TP	Training population
UAVs	Unmanned aerial vehicle
USA	United States of America
UZ Farm	University of Zimbabwe Farm

Published articles and submitted manuscripts pertaining to this PhD thesis

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Chapter 1

Gunundu, R., Shimelis, H., & Mashilo, J. (2023). Genomic selection and enablers for agronomic traits in maize (*Zea mays*): A review. *Plant Breeding*, *142*(5),573–593. https://doi.org/10.1111/pbr.13127

Chapter 2

Gunundu, R., Shimelis, H., & Tesfamariam, S.A. (2025). Genetic Diversity and Population Structure Analyses of Tropical Maize Inbred Lines Using Single Nucleotide Polymorphism Markers. PLoS ONE, *20*(1): e0315463. https://doi.org/10.1371/journal.pone.0315463

Chapter 5

Gunundu, R., Shimelis, H., & Tesfamariam, S.A. (2024). Combining Ability, Hybrid Prediction, and Genotype by Environment Interaction Analyses of Single-Cross Maize Hybrids Derived from Genomic Predicted Lines. Under review in *Euphytica*.

Introduction to thesis

Background of the study

Maize (*Zea mays* L., $2n = 2x = 20$) plays a vital role in global food security and economies. The crop is highly adaptable and grown in diverse agro ecological regions worldwide, serving multiple purposes such as food, feed, industrial applications, and bioenergy production. It is the second most widely cultivated cereal crop worldwide, after wheat, and provides approximately 30% of Africa's energy intake and is a staple food in many developing countries. Maize demand is projected to increase by 30% in sub-Saharan Africa (SSA) by 2050 due to population growth and urbanization (Ekpa et al., 2018). However, maize productivity in Africa remains low (< 2 t/ha) due to unavailability and limited access to seed of improved varieties, abiotic stresses such as drought and heat stress, flooding, waterlogging, erosion, and poor soil health, biotic stresses like diseases and insect pests, socioeconomic constraints, and policy issues (Cairns et al., 2013). There is need for a 2.4% annual increase in maize production and productivity and doubling of global output by 2050 to meet the demand for maize products (Ray et al., 2013). Developing resilient and high-yielding maize varieties is crucial to achieving the potential productivity of the crop that can reach up to 10 t/ha.

Hybrid maize breeding is essential for developing resilient, locally adapted, and high-performing cultivars. Hybrids offer numerous benefits, including increased yields, improved disease and pest resistance, and enhanced nutritional content. They also exhibit exceptional resilience to environmental challenges (Varshney et al., 2021). The success of hybrid breeding relies on heterosis, which is maximized by genetic recombination of genetically distant and contrasting inbred lines (Swarup et al., 2021). Therefore, a well-characterized genetic resource is crucial for identifying potential parents and heterotic groups that can guide maize breeding and conservation efforts (Reif et al., 2004; Giordani et al., 2019). Analyzing elite lines' genetic diversity and population structure is valuable to discerning the genetic relationships and selecting contrasting parents for hybrid breeding (Adu et al., 2019). Elite inbred lines are assigned to distinct heterotic groups using various markers, including DNA markers such as Single Nucleotide Polymorphism (SNP) markers, which are valued for their low cost, widespread presence on the genome, and amenability to high-throughput analysis (Mammadov et al., 2012; Semagn et al., 2012; Adu et al., 2019).

Single Nucleotide Polymorphism markers are ideal for genomic selection (GS), to optimize quantitative trait selection using genome-wide markers and historical phenotypic data (Heslot et al., 2012). The genotypic best linear unbiased prediction (GBLUP) model predicts selection response and genetic values using a genomic relationship matrix, enabling accurate predictions and efficient breeding (Habier et al., 2007; VanRaden (2008). Genetic estimated breeding values (GEBVs) and genetic estimated genetic values (GEGVs) are calculated for new individuals to discern additive and non-additive genetic variances and variety design. Genomic-assisted breeding can enhance yield and yield components in maize and related crops through precision and speed breeding (Schrag et al., 2008; Albrecht et al., 2011). GEBVs and GEGVs are used to rank and select inbred lines, clones, or families. Inbred lines with high GEBVs and GEGVs can accelerate superior hybrid development, such as single cross hybrids (SCHs) (Riedelsheimer et al., 2013).

Single cross hybrids have revolutionized maize productivity in the USA, China, Russia, Ukraine, and Brazil (Wang et al., 2011). Their resilience to diverse growing conditions and maximum heterosis make them ideal for high-input production conditions. Additionally, SCHs exhibit uniform characteristics and can buffer both adverse and optimal maize production environments (Joshi and Gautam, 2021). Determining the degree of heterosis is crucial for pipeline hybrid breeding and seed production. Combining ability analysis of elite inbred lines is essential to identify the best breeding parents and optimize hybrid development. Factorial mating designs play a vital role in determining good combiners and elucidating gene action and variance components (Wang et al., 2011). Combining ability analysis is critical in maize breeding as it enables the selection of desirable parental lines with the best general combining ability (GCA) and experimental hybrids with the best specific combining ability (SCA) effects (Lal et al., 2023). Several researchers have illustrated the impact of combining ability analysis in selecting maize genotypes for yield and component traits.

Multi-environment trials are crucial in breeding programs to establish specific and broad adaptations and recommend the best hybrid varieties (Devi and Singh, 2011). Genotype-by-environment interaction (GEI) analyses are crucial for identifying stable genotypes with consistent performance in specific or broad production environments (Yan and Tinker, 2006; Gauch et al., 2008). Various statistical methods, such as the additive main effects and multiplicative interaction (AMMI) model, genotype and genotype-by-environment interaction biplot analysis (GGE biplot), and stability and cultivar superiority indices (CSI), are used to analyze GEI (Lin and Binns, 1988; Yan and Tinker, 2006; Gauch et al., 2008). These methods help assess cultivar stability and performance, identify genotypes with broad and specific adaptations, and pinpoint top-performing genotypes. By leveraging these tools, breeders can develop hybrids that excel in various environments and contribute to food security.

Rationale of the study

Global food demand, population pressure, and climate change are the causes of food insecurity, necessitating the development of innovative strategies to enhance food production and supply (Mc Carthy et al., 2018). This is particularly crucial in Sub-Saharan Africa (SSA), where maize is a vital crop for millions of households. Maize productivity in SSA remains low (< 2t t/ha), far below the global average of 6 t/ha. Hence, developing new and innovative strategies that enhance maize production and productivity in SSA is crucial. Conventional breeding methods are time-consuming and costly. These methods can be complemented with speed breeding and genomic selection to design and commercialize high-performing new maize hybrids. Leveraging genomic selection and marker-assisted breeding can enhance the efficiency of hybrid maize development with high yields capable of thriving in diverse environmental conditions.

Seed Co Ltd has been developing tropical maize inbred lines using founder and derived lines from defined heterotic groups to enhance maize breeding efficiency and boost grain yields. The inbred lines must be rigorously evaluated and genetically differentiated using economic, agronomic traits and high throughput genetic markers to determine their genetic distance and composition. This involved characterizing test lines using diagnostic SNP markers to enable the selection of genetically distinct lines for marker-assisted hybrid maize breeding and the identification of beneficial alleles to maximize heterosis. Integrating genomic selection (GS) into the breeding program will reduce the slow development pace of hybrids and the high phenotyping costs. GS will predict the performance of advanced generations based on early-generation genotyping, using genetic estimated breeding values (GEBVs) and genetic estimated genetic values (GEGVs). This will identify genetically distinct inbred lines with good combining ability, informing selection decisions. Good combiner hybrids must be evaluated across targeted production environments using GEI analysis to assess their adaptability for cultivar selection and targeted deployment. Furthermore, the multi-environment evaluation of selected lines in hybrid combinations will assess gains in yield and yield components, guiding the selection of high-yielding and stable experimental hybrids for production and deployment.

Overall aim and objectives of the study

The overall research goal was to boost the grain productivity of maize by integrating phenotypic and genomic selections among a panel of new and founder maize inbred lines and single cross hybrids across target production environments in Zimbabwe and Zambia.

Specific objectives:

The specific objectives of the study are:

(i) to assess a maize germplasm panel's genetic diversity and population structure comprising 182 founder lines and 866 derived inbred lines using Single Nucleotide Polymorphism markers to identify genetically unique lines for hybrid breeding.

(ii) to predict maize grain yield and component traits using genome-wide markers and phenotypic data, based on the genomic best linear unbiased predictions model that accounts for additive and dominant genetic effects to estimate breeding values and guide the development of new inbred lines and hybrids.

(iii) to assess the gains in yield and yield components among single cross maize hybrids selected through genomic prediction across representative locations to guide breeding and production and

(iv) to determine the combining ability effects of newly selected inbred lines and quantify the magnitude of heterosis and genotype by environmental interaction (GEI) effects of single cross hybrids to select and recommend contrasting elite lines and experimental hybrids.

Research hypotheses

(i) The maize germplasm panel comprising 182 founder lines and 866 derived inbred lines will exhibit significant genetic diversity and population structure, allowing for the identification of genetically unique lines that can be used as parental lines for hybrid breeding.

(ii) Genome-wide prediction using SNP markers and phenotypic traits will accurately estimate genomic estimated breeding values and genomic estimated genetic values for yield and component traits in maize, enabling the identification of superior inbred lines and optimal parental combinations for hybrid breeding.

(iii) Single cross maize hybrids selected through genomic prediction will exhibit significant gains in yield and yield components across representative locations, validating genomic selection for maize improvement.

(iv) New inbred lines will show significant combining ability, and single cross hybrids will exhibit substantial heterosis and GEI effects, guiding the selection of elite lines and experimental hybrids for improved yield stability.

Thesis outline

The thesis consists of five chapters, each addressing a specific objective (as outlined in Table 0.1). Chapter 1 serves as a standalone literature review, while Chapters 2-5 are presented as separate research papers, each with its format and structure. Following these chapters is a general overview and implications of the findings. The literature review and the four experimental chapters have been condensed into distinct but interconnected papers, following the preferred thesis format of the University of KwaZulu-Natal. As a result of this structure, there may be some overlap and repetition of references and introductory information between chapters.

Table 0.1 Thesis structure

Chapter	Title
-	Thesis Introduction
1	Review of Literature
2	Genetic diversity and population structure analysis of tropical maize inbred lines using Single Nucleotide Polymorphism markers.
3	Genome-wide prediction of yield and component traits in maize using qualitative and quantitative phenotypic traits and genomic best linear unbiased prediction model
4	Response of single cross maize hybrids selected through genomic predictions for yield and yield Components
5	Combining ability, hybrid prediction, and genotype by environment interaction analyses of single-cross maize hybrids derived from genomic predicted lines
-	An overview of the research findings

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CHAPTER ONE

Literature Review: Genomic Selection and Enablers for Agronomic Traits in Maize (*Zea mays* L.): A review

Abstract

Maize (*Zea mays* L., 2n=2x=20) is a commodity crop providing millions of people with food, feed, industrial raw material and economic opportunities. However, maize yields in Africa are relatively stagnant and low, at a mean of <2 tons/ha compared to the global average of 6 tons/ha. The yield gap can be narrowed with accelerated and precision breeding strategies that are required to develop and deploy high-yielding and climate-resilient maize varieties. Genomic and phenotypic selection are complementary methods that offer opportunities for the speedy choice of contrasting parents and derived progenies for hybrid breeding and commercialization. Genomic selection (GS) will shorten the crop breeding cycle by identifying and tracking desirable genotypes and aid the timeous commercialization of market preferred varieties. The technology, however, has not yet been fully embraced by most public and private breeding programs, notably in Africa. This chapter aims to present the importance, current status, challenges and opportunities of GS to accelerate genetic gains for economic traits to speed up the breeding of high-yielding maize varieties. The first section summarizes genomic selection and the contemporary phenotypic selection and phenotyping platforms as a foundation for GS and trait integration in maize. This is followed by highlights on the reported genetic gains and progress through phenotypic selection and GS for grain yield and yield components. Training population development, genetic design and statistical models used in GS in maize breeding are discussed. Lastly, the review summarises the challenges of GS, including prediction accuracy and integrates GS with speed breeding, doubled haploid breeding and genome editing technologies to increase breeding efficiency and accelerate cultivar release. This will guide breeders in selection and trait introgression using GS to develop cultivars preferred by the marketplace.

Keywords: *Genomic selection, genetic gains, speed breeding, phenotypic selection, prediction accuracy*

1.0 Introduction

Maize (*Zea mays* L., 2n=2x=20) is an important crop globally serving for food, feed, bioethanol production and source of industrial raw material. Maize grain has unique nutritional value, particularly high starch content (e.g. amylose and amylopectin) which accounts for 65-75% per 100g of dry grain weight, fibre (7.3g/100g), protein (8-11g/100g), and fat (4%/100g). The grains are a vital source of vitamins (e.g. carotenoids, riboflavin, folate and niacin) and essential mineral nutrients such as magnesium, zinc, phosphorus, potassium, sodium, calcium and iron (Enyisi et al., 2014; Ignjatovic-Micic et al., 2015; Erenstein et al., 2022). Maize serves as an industrial raw material to produce starch, sweeteners, oil, drinks, glue and bio-ethanol (Adiaha, 2017; Serna-Saldivar & Carrillo, 2019; Erenstein et al., 2022).

In the continental Africa, maize is primarily grown for food. About 80% of the maize produced is consumed as human food (Prasanna et al., 2021). The fresh and dry maize stalks are used as animal feed because of their high digestibility, high biomass, palatability and high nutrient concentration. Maize stalk is rich in crude protein (9.01% on a dry matter basis), fiber (64.54%) and approximately 78.19% moisture content. These nutrient profiles make maize stalk suitable for use as livestock feed (Gao et al., 2019). Maize accounts for 40% of the total grain production in Africa, along with wheat and rice making it the continent's most widely produced cereal crop (Beyene et al., 2016; Prasanna et al., 2021).

In Africa, the total area under maize production is approximately 42 million hectares providing an estimated grain yield of 91 million tonnes per annum (FAOSTAT, 2024). Maize productivity in Africa, however, remains low (< 2 t/ha) compared to a global mean of 6 t/ha due to several abiotic production constraints, including drought and heat stress, flooding, waterlogging, poor soil fertility, soil erosion and poor soil health (Leitner et al., 2020; Santpoort, 2020; Thomas, 2020; Siatwiinda et al., 2021). Biotic stresses such as plant diseases (e.g., grey leaf spot, *Turcicum* leaf blight, leaf rust, maize streak virus and maize lethal necrosis), parasitic weeds (e.g. *Striga* species), and insect pests (e.g., stalk borer, grain weevils and the fall armyworm) contribute to significant yield losses or crop failure (Cairns et al., 2013; Beyene et al., 2017; Keno et al., 2018). Lack of improved maize varieties that possess adequate and durable tolerance to biotic and abiotic stresses is the cause of the overall yield loss. There is a need for an efficient and effective breeding approach that allows timeous development and release of improved varieties with desirable end user traits. Genomic selection (GS) offers opportunities for precision and speed breeding and can accelerate the development and release of high performing maize varieties (Heffner et al., 2009; dos Santos et al., 2016; Shikha et al., 2017). Genomic selection is one of the marker-assisted selection techniques based on genome-wide molecular markers and historical phenotypic data analyses to predict the performance of genetically differentiated individuals (Heslot et al., 2012). The success of GS is dependent on the availability of a training population (TP) developed through precision phenotyping of a set of maize lines for the traits of interest, followed by genotyping using highly discriminant molecular markers. Phenotyping can be done using aerial, ground and microscopic platforms. Molecular markers such as single nucleotide polymorphisms (SNPs) and simple sequence repeats (SSRs) have been widely used for genotyping. The data from the TP is utilized for training a statistical model that estimates the effect of each tested marker and the genomic estimated breeding values (GEBVs) for each individual for subsequent selection of genotypes (Xu et al., 2020). Several statistical models are used in GS, and the choice depends on the accuracy of the model and the genetic structure of the test genotypes. GS is helpful for the selection of essential traits, including grain yield, which has complex inheritance. GS guides the identification of the best parents based on an individual's GEBVs. Genomic estimated breeding values allow the selection of superior parents or individuals for next generation advancement faster than phenotyping alone (Xu et al., 2020). The benefit of GS is the ability to precisely forecast an individual's breeding values before it manifests a phenotype. The early selection decisions exclude undesirable individuals limiting expensive field tests. Hence, GS can increase genetic gains, shorten breeding cycles, and reduce breeding and germplasm management costs.

Reportedly, GS has provided a higher discriminative ability and increased grain yield responses two to four times higher than conventional phenotypic selection (Beyene et al., 2015). Additionally, GS is regarded as complementary to speed breeding approaches. GS enables three selection cycles in a single year (Beyene et al., 2015; Wang et al., 2015; Zhao et al., 2015; Watson et al., 2017; Hickey et al., 2019). The method can be combined with other modern breeding strategies (e.g. doubled haploid, gene editing, high throughput phenotyping and genotyping and speed breeding). This will enhance the response to selection and genetic gains for economic traits. Cabrera-Bosquet et al. (2012) recommended combining GS with high-throughput phenotyping methods to increase the efficiency of both methods. Combining GS and doubled haploid breeding and selecting the best haplotypes (i.e., optimal haploid value selection) predicts the best doubled haploid that can be produced from a segregating population. This increases genetic diversity and genetic gains in crop plants (Daetwyler et al., 2015).

Genomic selection has been successfully used in the selection of high performing maize germplasm with drought tolerance (Cooper et al., 2014; Zhang et al., 2015; Shikha et al., 2017). Key agronomic traits, including kernel numbers per row, row numbers per cob and grain yield, were successfully selected in maize using GS (Shikha et al., 2017). Widely grown Pioneer AQUAmax® line of drought tolerant maize hybrids in the USA have been developed using GS (Cooper et al., 2014).

Genomic selection has not yet been fully utilized in maize breeding programs, notably in Africa. This may be mainly due to the high costs of recurrent genotyping and phenotyping, lack of high-throughput phenotyping and genotyping facilities, poor infrastructure, lack of expertise and skilled staff turnover. Advanced genotyping and phenotyping technologies are required to offer precise genomic characterization, gene discovery and quantitative trait loci (QTL) mapping for breeding and genetic analysis. In light of the above background, this review aims to present GS's importance, current status, challenges and opportunities to accelerate genetic gains for economic traits and speed breeding of high-yielding maize. The first section summarizes genomic selection and the contemporary phenotypic selection and phenotyping platforms for genotype selection and trait integration in maize. This is followed by a summary on the reported genetic gains and progress through phenotypic selection and GS for grain yield and yield components. Training population development, genetic design and statistical models used in GS in maize breeding are presented. Lastly, the review discusses the challenges of GS, including factors affecting the prediction accuracy and the integration of GS with speed breeding, doubled haploid breeding and genome editing technologies to increase breeding efficiency and accelerate cultivar release. The review will guide breeders in selection and trait introgression using GS to develop cultivars preferred by the marketplace.

1.1 Genomic selection for agronomic traits in maize

Genomic selection (GS) is one of the marker-assisted selection techniques which uses genome-wide molecular markers and historical phenotypic data to predict the performance of genetically related individuals (Heslot et al., 2012). GS involves assembling a population of individuals of a known phenotypic and genotypic constitution (Crossa et al., 2013; Wang et al., 2018; Dreisigacker et al., 2021). GS is dependent on a training population from which a statistical model is built that links variation in the genotypes of the individuals' marker loci to variation in their observed phenotypes (Solberg et al., 2008; Hayes et al., 2009; Crossa et al., 2017). The statistical model obtained from the genotype and phenotype is then applied to a prediction population comprised of individuals for which only genotypes are available. Figure 1.1 summarises the general overview of GS by involving training population development and marker effects estimation and analysis of test populations to compute genomic estimated breeding values. When a hybrid's performance has not been field tested, either by the hybrid's parents or by previous hybrids derived from those parents, genomic prediction of maize hybrid performance makes it possible to discover prospective hybrids (Lorenz et al., 2011; Zhao et al., 2015; Chakradhar et al., 2017).

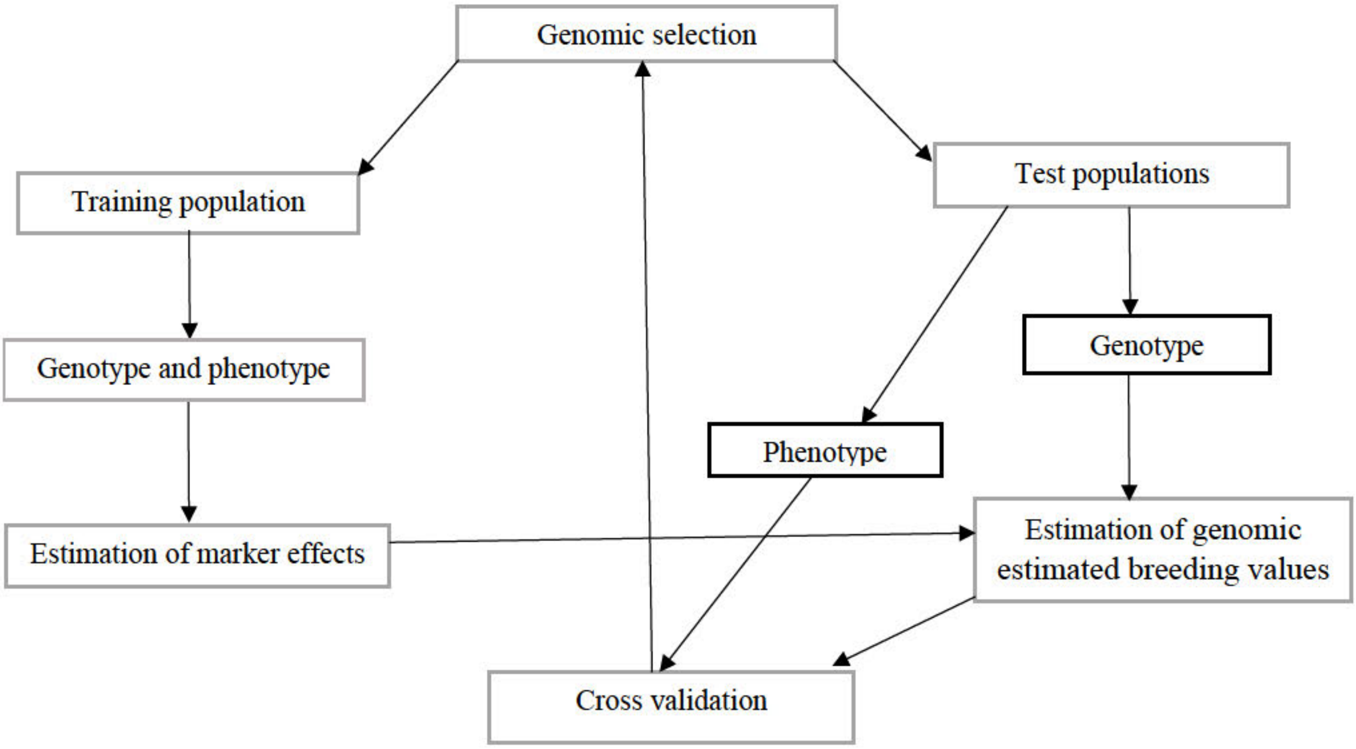

Figure 1.1 General overview of GS involving training population development and marker effects estimation and analysis of test populations to compute genomic estimated breeding values.

Table 1.1 presents some agronomic traits which have been successfully studied using GS, the number of genotypes studied, trait prediction accuracy obtained and marker systems used. Single nucleotide polymorphism (SNPs) is the most widely used marker system for GS in maize. Single nucleotide polymorphisms are differences found at the level of nucleotide base in the genome that distinguish individuals (Rafalski, 2002). They are the markers of choice in crop genetics and plant breeding applications because they occur in high abundance in genomes. There is also a wide range of genotyping platforms with variable capabilities to carry out SNP analysis (Rafalski, 2002).

Prediction accuracy is the measure of how reliable a future phenotype of target individuals can be predicted based on the correlation between the GEBVs and empirically estimated breeding values (Lorenz et al., 2011). The correlation directly links the GEBV forecast accuracy to the selection response and measures selection accuracy. The magnitude of prediction accuracies are classified as low (0.1 to 0.4), medium (0.4 to 0.6) and high (0.6 and above) (Môro et al., 2017; Shikha et al., 2017; Beyene et al., 2021;Yong et al., 2021). Low prediction efficiencies were reported for grain yield in Brazil (0.26), China (0.24) and Germany (0.42) (Table 1.1). The low accuracies are attributable to the population's genetic compositions, the marker system used, the genetic architecture of the trait, the statistical method used, and the trait's heritability (Zhang et al., 2019). Conversely, high prediction accuracies were reported for grain yield in India (0.98), Mexico (0.93), Kenya (0.68 to 0.77) and the USA (0.61 to 0.87) (González-Camacho et al., 2012; Massman et al., 2013; Shikha et al., 2017; Santantonio et al., 2020; Beyene et al., 2021). Prediction accuracies greater than 0.8 were recorded for yield-related traits, including silking date, plant height, stalk lodging, ear diameter, ear length, dry matter accumulation and 1000 kernel weight in India, Mexico, Germany, USA and Kenya (Table 1.1). The high prediction accuracies may also relate with the use of appropriate statistical methods, optimum marker densities and test populations, minor allele frequency and heritability of the target traits. Reportedly, maize researchers in India recorded the most consistent prediction accuracies ranging between 0.84 and 0.99 for traits including anthesis-to-silking interval, ear length, kernel rows per cob and 1000 kernel weight (Table 1.1). Conversely, Chinese breeders reported lower prediction accuracies ranging from 0.19 (e.g. kernel dehydration rate) to 0.39 (e.g. grain moisture content) (Table 1.1).

Genomic selection has resulted in the development of maize hybrids with yield improvement and better adaptation to biotic and abiotic stresses. For example, 78 AQUAmax hybrids were developed through GS and deployed in the USA (Cooper et al., 2014). The hybrids have significantly higher yields under both favourable and drought-stressed conditions based on extensive field evaluation and data analysis. Further, the use of GS has resulted in the speed breeding of inbred lines with high general and specific combining abilities allowing hybrid variety development (Massman et al., 2013; Beyene et al., 2015a). The genomic estimation of breeding values has also been beneficial in identifying maize inbred lines with resistance to major diseases such as grey leaf spot, northern corn leaf blight and maize streak virus (Rice & Lipka, 2021). Therefore, GS technology facilitates the rapid selection of superior genotypes and accelerates the breeding cycle. Genomic-enabled prediction facilitates adequate germplasm characterization and selection and could accelerate the integration of essential traits to dispatch market preferred varieties.

Table 1.1 Genomic selection for agronomic traits, marker systems and prediction accuracy in maize

Country	Traits	Test genotypes	Marker system	Prediction accuracy	References
India	Anthesis to silking interval	240 subtropical maize lines	SNPs	0.84 - 0.97	Shikha et al. (2017)
	Ear length			0.84 - 0.99	
	Ear diameter			0.84 - 0.98	
	Rows per cob			0.85 - 0.98	
	Kernel rows per cob			0.86 - 0.98	
	1000 kernel weight			0.86 - 0.99	
	Grain yield			0.78 - 0.98	
Mexico	Anthesis date	504 doubled haploid and 296 inbred lines	SNPs	0.59	Crossa et al. (2013)
	Anthesis to silking interval			0.59	
	Grain yield			0.52	
	Anthesis date	300 tropical inbred lines	SNPs	0.67	González-Camacho et al. (2012)
	Silking date			0.34	
	Anthesis to silking interval			0.67	
	Grain yield			0.93	
Germany	Silking date	285 inbred lines	SNPs	0.81	Riedelsheimer et al. (2013)
	Plant height			0.72	
	Starch content			0.72	
	Sugar content			0.73	
	Lignin content			0.80	
	Dry matter concentration			0.80	
	Dry matter yield			0.78	
	Anthesis date	300 CIMMYT lines, 31 parental inbred lines, 1380 DH lines	SNPs	0.79	Albrecht et al. (2011)
	Grain yield			0.42	
	Grain dry matter content			0.60 - 0.80	
	Grain dry matter yield			0.50 - 0.70	
	Basal height	570 lines from testcrosses of 285 inbred lines	SNPs	0.49	Riedelsheimer et al. (2013)
	Sugar content			0.69	
Uganda	Fall armyworm damage	358 lines	SNPs	0.70 - 0.84	Badji et al. (2021)
China	Stalk length	2 recombinant inbred line populations	SNPs	0.30	Li et al. (2020)
	Days to anthesis	Single-cross maize population		0.30	Yong et al. (2021)
	Ear height			0.32	
	Grain moisture content			0.39	
	Kernel dehydration rate			0.19	
	Grain yield			0.24	
United States of America	Plant height	Testcrosses, backcrosses and synthetics	SSR, RFLP	0.70	Lorenzana & Bernardo (2009)
	Ear height			0.49	
	Root lodging			0.70	

Table 1.1 continued

Country	Traits	Test genotypes	Marker system	Prediction accuracy	References
Table 1.1 continued	Stalk lodging			0.26	
	Stover index			0.70	
	Grain moisture			0.58	
	Grain yield			0.61	
	Glucose content (g kg^{-1} dry matter)			0.69	
	Lignin content (% cell wall)			0.54	
	Male flowering, Anthesis to silking interval	4699 recombinant inbred lines from 25 bi-parental populations	SNPs	0.82	Guo et al. (2012)
	Starch content	257 inbred lines from four subpopulation	SNPs	0.34	Guo et al. (2014)
	Protein content			0.29	
	Oil content			0.42	
	Root lodging	479 single crosses	SNPs	0.69	Massman et al. (2013)
	Stalk lodging			0.84	
	Moisture			0.90	
	Grain yield			0.87	
Kenya	Plant height	849 doubled haploid lines	SNPs	0.50	Santantonio et al. (2020)
	Grain moisture			0.70	
	Grain yield			0.68	
	Anthesis date	3068 tropical maize doubled haploid lines	Repeat Amplification Sequencing (rAmpSeq)	0.65 - 0.76	Beyene et al. (2021)
	Plant height			0.68 - 0.80	
	Grain yield			0.62 - 0.77	
Brazil	Anthesis date	F_2 populations	SSR	0.28 - 0.53	Môro et al. (2017)
	Silking date			0.36 - 0.62	
	ASI			0.23 - 0.52	
	Plant height			0.25 - 0.37	
	Ear height			0.24 - 0.46	
	Plant lodging			0.27 - 0.35	
	Grain yield			0.13 - 0.26	

Abbreviations: SNPs, Single Nucleotide Polymorphism; SSR, Simple Sequence Repeats; RFLP, Restriction Fragment Length polymorphism; CIMMYT, International Maize and Wheat Improvement Centre

1.2 Enablers of genomic selection

1.2.1 Phenotyping of maize for agronomic traits

Phenotyping maize genetic resources is crucial in determining the extent of genetic variation and identifying sources of valuable traits for hybridization, QTL mapping and molecular marker design and development and breeding. High quality phenotypic data is essential for GS in crop breeding programs (Desta & Ortiz, 2014). Key agronomic traits for phenotyping maize genetic resources are presented in Table 1.2. Plant height, ear aspect, ear length and diameter, ear weight, kernel row number, kernel depth and size, shelling percentage and grain yield show wide phenotypic variation in maize (Table 1.2).

Plant height and ear height are highly and positively correlated with grain yield. Reportedly, taller genotypes with high ear placement have resulted in higher grain yields (Carvalho et al., 2017; Belay, 2018; Aman et al., 2020). The high yield is attributed to the high dry matter accumulation and mobilisation attributed to the high number of leaves per plant. Also, traits including plant stand, leaf area index, ear aspect, number of ears per plant, ear length, the number of kernel rows per ear, above-ground biomass, shelling percentage and 1000-kernel weight are positively correlated and confer a high positive direct effect on grain yield (Rafiq et al., 2010; Adu et al., 2016). The above selection procedure saves breeding costs and allows a simultaneous selection of economic traits to enhance grain yield rather than aiming for genomic selection for each trait.

Root length and root dry weight also correlate highly and positively with grain yield. Deep roots extract more soil water resulting in an extended duration of the green leaf area and an increase in grain yield. Longer roots reach the deeper soil horizon when moisture is limiting. Deep root systems resulted in plant growth and development and higher yields (Lilley and Kirkegaard, 2011; Abdel-Ghani et al., 2012). High root dry weight is essential for efficient soil water use and maintenance of root shape under moisture stress (Abdel-Ghani et al., 2012).

Days to 50% tasselling and silking correlate significantly and negatively with grain yield (Anjorin & Ogunniyan, 2014; Mogesse, 2022). This suggests that the two traits should be selected using proxy traits showing high heritability to improve yield gains. Selection for a high number of grains per ear may increase grain yield in maize (Nastasić et al., 2010). Therefore, selecting agronomic traits highly correlated with grain yield is required for the successful application of GS to improve maize productivity.

Table 1.2 Important agronomic, physiological and root traits for phenotyping maize genetic resources for genome-wide association studies

Trait	**Detected genetic variation**	**Correlation with grain yield**	**References**
Agronomic traits			
Anthesis date	Low - High	Low - High	Kumar et al. (2014)
Silking date	Low - High	Low - High	Pandey et al. (2017)
Plant height	High	High	Aman et al. (2020)
Ear height	High	Medium - High	Aman et al. (2020)
Grain filling period	Medium - High	Low - High	Golam et al. (2011)
Days to maturity	Medium - High	Medium - High	Belay (2018)
Ears per plant	Low	High	Carvalho et al. (2017)
Ear aspect	Medium - High	Low - Medium	Pandey et al. (2017)
Ear diameter	High	High	Belay (2018)
Ear length	High	High	Belay (2018)
Ear/cob weight	High	High	Pandey et al. (2017)
Kernel rows per ear	High	High	Pandey et al. (2017)
Kernel numbers per row	High	Medium - High	Carvalho et al. (2017)
Kernel depth	High	High	Nastasić et al. (2010)
Kernel size	High	High	Boćanski et al. (2009)
1000 kernel weight	High	High	Aman et al. (2020)
Field weight	High	High	Shakoor et al. (2007)
Shelling percentage	High	High	Pandey et al. (2017)
Moisture content of grain	High	Low - Medium	Nzuve et al. (2014)
Grain yield	High	High	Shakoor et al. (2007)
Physiological traits			
Biomass accumulation	High	High	Golam et al. (2011)
Leaf area index	High	High	Orlando et al. (2016)
Canopy height	High	High	Jimenez-Berni et al. (2018)
Canopy structure	Medium - High	Medium	Sankaran et al. (2015)
Chlorophyll content	Medium - High	High	Sankaran et al. (2015)
Disease reaction			
Diplodia ear rots	Medium - High	High	Nzuve et al. (2014)
Fusarium ear rots	Medium - High	High	Nzuve et al. (2014)
Root traits			
Root lodging	Low	Low - Medium	Nzuve et al. (2014)
Root length	Low - High	High	Abdel-Ghani et al. (2012)
Root dry weight	Low - High	High	Abdel-Ghani et al. (2012)

1.2.2 Phenotyping platforms

Various emerging precision phenotyping platforms have become available for crop phenotyping. High throughput phenotyping is vital to improving selection efficiency, assessing plant health and guiding crop management practices (Song et al., 2021). Automatic, efficient and precise phenotyping methods are critical for comparative selection involving phenotypic and genomic data (Wang et al., 2019; Song et al., 2021).

Table 1.3 presents some of the phenotyping platforms that have gained prominence for maize germplasm evaluation. The platforms are broadly categorized into three, namely: ground based, aerial and microscopic. Under these categories, phenotyping can be done at (1) individual plant and organ levels measured *ex-situ,* (2) plot and canopy level measured *in-situ* and (3) microscopic level (Wang et al., 2019; Song et al., 2021). The common phenotyping platforms are briefly summarised below.

1.2.2.1 Ground phenotyping

Ground based phenotyping platforms can be divided into two categories, namely portable and stationary. The method involves instruments such as smartphones, cameras (Asaari et al., 2019), maize ear scanners (Friedli et al., 2016; Sunil et al., 2018), robots (Bai et al., 2019) and tractors fixed with cameras (Song et al., 2021). Traits such as plant and ear height, leaf area index, leaf length, leaf area, leaf size, ear size, number of kernels, kernel size, plant biomass and root length have been successfully phenotyped using this method (Orlando et al., 2016; Brichet et al., 2017; Bai et al., 2019; Yang et al., 2020).

Ear parameters such as height and size, kernel size and the number of kernels were phenotyped by various workers (Bai et al., 2019), Brichet et al. (2017) and Warman et al. (2021). Brichet et al. (2017) used a robot assisted imaging method to phenotype maize ear growth and silk development. Friedli et al. (2016) used terrestrial laser scanning (TLS) to phenotype canopy development in maize. Qiao et al. (2019) phenotyped 174 maize genotypes for total root length and total shoot length using a semi–hydroponic phenotyping technique. This helped to select maize genotypes with improved adaptation to different target environments. The LeasyScan method is one of the popular methods used for phenotyping plant height, total leaf area, leaf area index (LAI), leaf area development, water-use efficiency and canopy transpiration rate in maize (Sunil et al., 2018). The LeasyScan can be used to phenotype traits controlling plant water use with high throughput and efficiency. This helped in selecting improved varieties of maize with good drought adaptation (Vadez et al., 2015; Sunil et al., 2018).

1.2.2.2 Aerial phenotyping

This method mainly uses unmanned aerial vehicles (UAVs) and satellites to phenotype plant canopy structure, plant height, chlorophyll content, nitrogen content and above ground plant biomass (Jang et al., 2020; Lobell et al., 2020; Song et al., 2021). Also, the method measures LAI, number of leaves and leaf size. Liebisch et al. (2015) used a Zeppelin NT aircraft to phenotype 16 maize lines for plant density, plant vigour, leaf size, LAI, leaf biomass and radiation interception. Yang & Hoffmann (2015) used an Air Tractor (AT-402B) installed with a camera, GPS receiver and video monitor to measure plant biomass in maize. Unmanned aerial vehicles and vehicle-mounted phenotyping methods have been used for large-scale phenotyping. Satellite images have also been used to study the growth and genetic differences among crop varieties in the field on a large scale. This technique is nondestructive and involves low cost and user-friendly devices, which are critical for high-throughput phenotyping in plant breeding programs.

1.2.2.3 Microscopic phenotyping

This method involves phenotyping at cellular and tissue levels to enhance the genotype and phenotype relationships (Zhang et al., 2018). Three-dimensional (3D) models of kernel geometry based on microcomputed tomography (micro-CT) have been developed for maize microscopic phenotyping (Hughes et al., 2017; Wu et al., 2019). The method has greatly improved the precision of phenotyping interior tissue structures of maize kernels, stalks and leaves, allowing the detection and quantification of fungal and bacterial pathogens (Du et al., 2016). Microscopic pathogens affect plant growth, grain yield and quality. Minker et al. (2018) reported using a 3D phenotyping platform to decipher cellular features underlying variation in disease development, such as gray leaf spot (*Cercospora zea maydis*). Field phenotyping is widely practiced in public-sector maize breeding programs, which is limiting higher genetic gain. Field phenotyping must be augmented with GS to enhance the accuracy, efficiency and quality of data collection and enhance selection response.

Table 1.3 Ground, aerial and microscopic phenotyping methods and their uses in maize breeding

Phenotyping platform	Features	Traits measured	References
Ground based phenotyping: - (High resolution smart cameras, RGB cameras, robots, photosynthesis sensors, stereo cameras, thermal cameras, multi/hyperspectral cameras, incident light sensors, scanners, thermal infrared imaging cameras, semi-hydroponic phenotyping platforms)	Applied for field plots and provide high resolution data	Leaf area index	Orlando et al. (2016); Shafiekhani et al. (2017)
		Canopy development across growth stages	Virlet & Sabermanesh (2016)
		Canopy height	Jimenez-Berni et al. (2018)
		Plant height Ear height Leaf width Leaf length Leaf area Leaf size Leaf moisture content Plant biomass	Bai et al. (2019); Miao et al. (2020); Wang et al. (2018); Yang et al. (2020)
		Tassel size	Lu et al. (2017)
		Flowering time	Leiboff et al. (2015)
		Growth rate of ear and silk Ear size	Brichet et al. (2017)
		Shoot and root growth	Le Marié et al. (2016)
		Drought tolerance	Asaari et al. (2019)
		Number of kernels Kernel size	Warman et al. (2021)
		Total shoot length Total root length	Qiao et al. (2019)
		Leaf area Water use efficiency	Sunil et al. (2018)
Aerial phenotyping (e.g. satellites and unmanned aerial vehicles (UAVs)	Used under field conditions with high and efficient resolution	Plant height Canopy size Chlorophyll content Plant biomass	Li et al. (2015); Sankaran et al. (2015)
		Leaf stay green Leaf rolling Drought tolerance	Trachsel et al. (2019)
		Grain yield under nitrogen stress	Zaman-Allah et al. (2015)

Table 1.3 continued

Phenotyping platform	**Features**	**Traits measured**	**References**
		Plant density Plant vigour Leaf size Leaf area index Leaf biomass	Liebisch et al. (2015)
Microscopic phenotyping	Used at cellular and tissue levels with low imaging resolution	Leaf number Stalk lodging Kernel size Kernel weight Kernel composition	Zhang et al. (2018)
		Seedling shoot apical meristem size Stem size	Leiboff et al. (2015)
		Root size	Song et al. (2021)
		Stalk size	Du et al. (2016)
		Disease resistance Plant- pathogen interactions	Minker et al. (2018)

Abbreviations: RGB camera, red, green and blue wavelength camera; UAVs, Unmanned aerial vehicles

1.3 Genetic gain

Assessing genetic gains for yield and yield component traits is an essential step in maize breeding programs to devise effective breeding strategies and advance germplasm with favourable traits. Genetic gains for maize yield and key agronomic traits across different countries are presented in Table 1.4.

Higher gains in grain yield have been reported in Nigeria, South Africa, China, Brazil and the United States of America. Higher gains were the result of the continuous development and deployment of high-yielding maize hybrids, which possess better agronomic and physiological traits associated with higher yield potential. Aylward et al. (2015) reported that maize yields in Malawi tripled between 2005 and 2007, from 810 to 2680 kg/ha, while in Rwanda, by threefold between 2007 and 2011, from 720 to 2340 kg/ha due to the use of improved and locally adapted maize varieties and improved agronomic practices. Beyene and Prasanna, (2020) reported genetic gains in intermediate maturing maize varieties in East and Southern Africa (ESA) by 3.28% between 2008 and 2013, and by 1.75% between 2013 and 2017 under optimal growing conditions. The gains were attributed to tolerance to biotic and abiotic stresses. Masuka et al. (2017) reported yield gains under different growing conditions within CIMMYT derived maize hybrids from 2000 to 2010 in ESA. The authors pinpointed that under optimum, managed drought, random drought, low nitrogen and maize streak virus conditions, the gains ranged from 20.9 to 141.3 kg ha^{-1} year $^{-1}$ in Kenya, Malawi, South Africa, Uganda, Zambia and Zimbabwe. Badu Apraku et al. (2015) reported average gains in grain yield of 13.5 kg ha $^{-1}$ year $^{-1}$ under drought, 41 kg ha $^{-1}$ year $^{-1}$ under *Striga* conditions and 30 kg ha $^{-1}$ under combined drought, *Striga* and low nitrogen conditions in Nigeria.

The use of improved varieties in Ethiopia has resulted in significant yield gains varying from 1500 to 3230 kg/ha between 2004 and 2013 (Abate et al., 2015). The adoption of hybrid varieties, such as BH661 and BH546 contributed to increased yield gains in Ethiopia. In the USA, grain yields increased at a rate of 115 kg ha^{-1} year $^{-1}$ from 1934 to 2004. Considerable yield advances varying from 2500 and 7500 kg ha^{-1} at a rate of 80 kg ha^{-1} year $^{-1}$ have been recorded in Canada between 1940 and 2000. Between 1986 and 2012, Serbia experienced maize yields gains of 22 kg ha^{-1} year $^{-1}$. Additionally, between 1964 and 2001, China had genetic gains of approximately 60 kg ha^{-1} year $^{-1}$. The adoption of superior cultivars and improved cultural practices have been largely credited for the reported gains (Duvick, 2005; Lee & Tollenaar, 2007; Wang & Bubeck, 2011; Videnović et al., 2013).

Under stress environments, significant yield gains were associated with reduced anthesis-silking-interval (ASI), improved stalk and root lodging, good plant and ear aspects, a high number of ears per plant, a high number of kernels per ear, increased plant and ear height and productive number of ears per plant (Badu-Apraku et al., 2014; Maphumulo et al., 2015; Abdulmalik et al., 2017; Sun et al., 2017; Oyekunle et al., 2019). Under optimal growing conditions, genetic gains in plant and ear height, husk cover, root and stalk lodging tolerance, plant and ear aspects, number of ears per plant, a higher number of kernels per ear and higher kernel weight contributed to increased grain yield (Badu-Apraku et al., 2014; Al-Naggar et al., 2016; Abdulmalik et al., 2017; Sun et al., 2017; Oyekunle et al., 2019). Also, improved management practices (e.g. optimum plant population, crop rotation, optimum sowing depth, right planting time, weeding, controlling insect pests and diseases and fertilizer management) bolstered maize yields globally (Qian et al., 2016; Liu et al., 2017). The effectiveness of technologies and innovations in maize breeding programs must be evaluated through regular assessments of genetic gains. This will enable to devise effective breeding strategies and measure socio-economic impacts.

Table 1.4 Relative genetic gain of grain yield and other agronomic traits of maize under stressed and non-stressed conditions

Country	**Trait**	**Relative genetic gain (% per year) under stress environments**	**Relative genetic gain (% per year) under non-stressed environments**	**References**
Nigeria, Ghana, Benin	Anthesis days	0.11	0.08	Oyekunle et al. (2019)
	Silking days	0.08	0.05 0.11	Badu-Apraku et al. (2014)
			0.07	Abdulmalik et al. (2017)
	Anthesis to silking interval	-0.05	0.03 0.79	Abdulmalik et al. (2017); Oyekunle et al. (2019)
	Plant height	0.22 0.90	0.28 0.9	Badu-Apraku et al. (2014)
	Ear height	0.01 0.18	0.38	Badu-Apraku et al. (2014); Oyekunle et al. (2019)
	Root lodging	-0.01	0.25	Badu-Apraku et al. (2014)
	Stalk lodging (%)	0.09	-0.38	Badu-Apraku et al. (2014)
	Plant aspect	-0.94	1.06	Oyekunle et al. (2019)
	Husk cover	-0.44	2.84	Badu-Apraku et al. (2014)
	Ear aspect	-0.51	-0.40	Badu-Apraku et al. (2014)
	Ears per plant	0.32 0.75	-1.00 0.24 0.69	Oyekunle et al. (2019)
	Grain yield	1.59 1.93	1.24 1.93	Badu-Apraku et al. (2014)
China	Anthesis to silking interval	0.04	0.041	Sun et al. (2017)
	Plant height	0.33	0.36	
	Empty ears/plant	-0.40	-0.31	
	Kernels per ear	2.01	1.97	
	Anthesis days		-0.15	Yong et al. (2021)
	Ear height		-1.42	
	Water content of ears		-0.64	
	Kernel dehydration rate		1.89	
	Grain yield		1.30	
Egypt	Plant height		0.59	Al-Naggar et al. (2016)
	Ears per plant		0.20	
Brazil	Anthesis days		-0.29	Olivoto et al. (2021)

Table 1.4 continued

Country	Trait	Relative genetic gain (% per year) under stress environments	Relative genetic gain (% per year) under non-stressed environments	References
	Silking days		-0.53	
	Plant height		3.31	
	Ear height		-0.47	
	Tassel length		0.16	
	Kernel depth		-0.27	
	Grain yield		4.97	
Argentina	Kernels per ear		2.98	Luque et al. (2006)
South Africa	Anthesis days		2.69	Maphumulo et al. (2015)
			0.18	Musundire (2021)
	Silking days		2.12	Maphumulo et al. (2015)
	Anthesis to silking interval		0.57	Maphumulo et al. (2015)
	Ear height		0.10	Musundire (2021)
	Root lodging		0.01	Musundire (2021)
	Stalk lodging (%)		0.32	Musundire (2021)
	Ear length		-0.67	Maphumulo et al. (2015)
	Ears per plant		0.20	Maphumulo et al. (2015)
	Grain yield		1.12	Maphumulo et al. (2015)
			1.46	Musundire (2021)
United States of America	Anthesis to silking interval		2.50	Campos et al. (2006)
	Ears per plant		0.50	
	Kernels per ear		2.00	
	kernel weight		0.21	
	Grain yield		0.20	

1.4 Training populations for genomic selection

Assembling a training population (TP) is critical for successfully implementing GS in maize breeding programs. A TP is a group of closely related individuals with known ancestries, such as half-sibs, full-sibs, doubled haploid lines and populations (Krishnappa et al., 2021). Designing the TP depends on the population structure and genetic lineages within and between genotypes. Training population development begins with assembling breeding lines which are then phenotyped for the traits of interest, followed by genotyping them using highly discriminative molecular markers such as single nucleotide polymorphisms (SNPs) (Jannink et al., 2010; Heslot et al., 2012; Massman et al., 2013). Marker effects are estimated using the TP's phenotypes and a prediction equation for the target trait (Lorenzana & Bernardo, 2009; Heffner et al., 2010). The selected candidates are genotyped, and the prediction equation is applied to calculate their genomic estimated breeding values (GEBVs) on which selection is based (Heffner et al., 2010; Heslot et al., 2015).

The number of markers (also referred to as marker density) plays a vital role in prediction accuracy. Increasing the number of markers improves QTL linkage analysis and improves prediction accuracy. There is an increased likelihood that each QTL will be in high linkage disequilibrium (LD) with at least one marker (Calus et al., 2008; De Roos et al., 2009; Goddard, 2009). De Roos et al. (2009) reported a low prediction accuracy of 0.07 without genetic markers. However, when using 300 markers, the prediction accuracy increased to 0.11. Accuracy increased to 0.18 when the marker density was 18000. The prediction accuracy of candidate trait selection was enhanced by increasing the number of TP individuals, which allowed accurate calculation of marker effect estimates (Liu et al., 2018; Norman et al., 2018; Zhang et al., 2017).

The size of the training population is vital when using GS in breeding. Optimizing the TP size and its association with the breeding or validation set improves the prediction accuracy of genomic prediction models (Zhang et al., 2017; Akdemir & Isidro-Sánchez, 2019; Sarinelli et al., 2019). Daetwyler et al. (2013) showed that increasing the TP population from 500 to 2000 improved GEBVs accuracy by 50%. Sarinelli et al. (2019) reported that increasing the TP size from 50 to 350 increased GEBVs accuracy for plant height from 0.56 to 0.68. Zhang et al. (2017) pinpointed that increasing the TP size from 1000 to 4000 enhanced the prediction accuracy for grain yield from 0.5 to 0.75.

Trait heritability impacts GS prediction accuracy in maize populations (Daetwyler et al., 2008 ; De Roos et al., 2009 ; Isidro et al., 2015). Higher heritability improves prediction accuracy (Zhang et al., 2017). The correlation between heritability and prediction accuracy was significant for trait-environment combinations, with highly heritable combinations showing higher prediction values (De Roos et al., 2009). The authors pinpointed that low heritability results in low prediction accuracy. A heritability of 0.10 led to a prediction level of 0.27, while a heritability greater than 0.50 increased accuracy to 0.55. Further, Liu et al. (2018) noted that higher heritability resulted in higher prediction accuracy for grain yield. For instance, an accuracy of 0.40 was recorded with a heritability value of 0.68, 0.71 and 0.94.

The prediction accuracy was reportedly enhanced among genetically related TP (Daetwyler et al., 2013). Genetically related breeding populations have more accurate GEBV values than distant populations. Differences between individual members of the training and testing sets also reduce the degree of LD between markers and QTLs. Albrecht et al. (2011) reported prediction accuracies of greater than 0.70 when using TPs with high genetic relatedness, but lower prediction accuracies of less than 0.50 were found when using TPs with low genetic relatedness. When the training set used 1000 individuals from a generation close to the test set, GEBV accuracy was higher (0.63 than 0.56) than when the same number of genetically distant individuals were used as the test set (Albrecht et al., 2011). The accuracy of prediction models and GS are dependent on training populations. There is a need for reliable, timely, cost-effective and repeatable phenotyping tools to develop training populations to advance GS.

1.5 Statistical models

Maize genomic selection is accelerated using various statistical models (McGaugh et al., 2021). The models identify high-merit individuals for a combination of traits. Statistical models are categorized into parametric and non-parametric. Parametric models include ridge regression best linear unbiased prediction (RR-BLUP), genomic best linear unbiased prediction (GBLUP), Bayes A, Bayes B, Bayes C, Bayes Cπ, least absolute shrinkage and selector operator (LASSO), bayesian least absolute shrinkage and selector operator (Bayes LASSO), least squares regression (LSR), Bayesian ridge regression (BRR) and ridge regression (RR). The models integrate various gene actions such as dominance, additive-by-additive, dominance-by-dominance, additive-by-dominance, and dominance-by-additive (Heslot et al., 2012; de los Campos et al., 2013; Lin et al., 2014; Isidro et al., 2015). Non-parametric models include support vector machine regression (SVM), reproducing kernel Hilbert space (RKHS), random forest (RF), Nadaraya–Watson estimator, Bayesian regularized neural networks (BRNN) and Radial basis function neural networks (RBFNN). They effectively predict phenotypes for inter-locus interactions and genotypic values that are influenced by dominance and the environment. Table 1.5 summarises various parametric and non-parametric models used in GS in maize.

Accurate predictions are critical in GS. Different statistical models have been used, yielding different results (Table 1.6). Hence, choosing the best model is crucial for GS. Factors such as population genetic structure and trait inheritance affect prediction accuracy. Lorenzana & Bernardo (2009) used multiple maize populations and the RR-BLUP and Bayesian methods to predict grain yield. The two models reportedly provided accurate results. Schulz-Streeck et al. (2012) pointed that the RR-BLUP model was highly accurate in predicting grain moisture content on 568 maize lines. The model had better resolutions than the LASSO model and was comparable to the RR and elastic net models.

Various genetic models have been utilized in maize breeding to predict trait inheritance, including GBLUP, RR-BLUP, MLR, Elastic Net, Bayes A, B, C and Cπ, RKHS, BRR, SVM, and BLR (dos Santos et al., 2016; Shikha et al., 2017; Liu et al., 2018; Badji et al., 2021). Several studies have utilized inbred lines, hybrids, and populations of varied generations to achieve high accuracy predictions using different models. For example, Badji et al. (2021) achieved prediction accuracies of 0.77 to 0.79 while predicting the selection of maize resistance to fall armyworm with BL, Bayes A, B, and C, RKHS, RR-BLUP, BRR, and BLR (Table 1.6). The models provided similar prediction accuracies. Heslot et al. (2012) used RR-BLUP, Bayes C, RKHS, SVM, Elastic Net, RF, and BL models to predict grain moisture content and yield among maize inbred lines with accuracy ranging from 0.32 to 0.79. The SVM model had the lowest (0.32 to 0.45), while the RKHS had the highest (0.64 to 0.79) accuracies for grain moisture and yield. Low prediction accuracies below 0.50 were recorded by Liu et al. (2018) in predicting grain yield in maize inbred lines. The authors reported accuracies that ranged from 0.38 to 0.40 using the RR-BLUP (0.38), GBLUP (0.39), Bayes A (0.39), Bayes B (0.40) , Bayes C (0.39), and RKHS (0.39) when predicting grain yield. Diverse statistical techniques have been developed to guide GS. Statistical tools enable complex data analyses using linear models and machine-learning algorithms to make inferences and selection decisions. For instance, kernel-based statistical tools like the RKHS have consistently produced accurate genomic predictions.

Table 1.5 Genomic selection models and nature of population and size in maize

Model	**Population**	**References**
RR-BLUP	238 inbred lines	dos Santos et al. (2016)
	930 inbred lines	Zhao et al. (2013)
	441 hybrids	Guo et al. (2012)
	285 diverse inbred lines	Riedelsheimer et al. (2012)
	255 inbred lines, 150 hybrids	Windhausen et al. (2012)
	788 lines from 4 inbred lines	Zhao et al. (2013)
	4699 lines from 25 crosses	Guo et al. (2012)
	294 lines and 441 hybrids	Guo et al. (2012)
G-BLUP	100 dent & 97 flint hybrids	Technow et al. (2013)
	635 lines, 5 DH populations	Riedelsheimer et al. (2013)
	3273 lines	Zhang et al. (2015)
	31 parents, 1380 DH lines	Albrecht et al. (2011)
	296 inbred lines	Crossa et al. (2013)
	504 DH lines	Crossa et al. (2013)
	1062 DH/F2	Lorenzana & Bernardo (2009)
RKHS (Reproducing kernel Hilbert space)	504 DH lines	Crossa et al. (2013)
	296 inbred lines	Crossa et al. (2013)
	300 inbred lines	González-Camacho et al. (2012)
	300 inbred lines	Crossa et al. (2011)
	300 inbred lines	Crossa et al. (2010)
	240 subtropical lines	Shikha et al. (2017a)
Bayes A, B	240 subtropical lines	Shikha et al. (2017a)
	4699 lines from 25 crosses	Guo et al. (2012)
BL (Bayesian least absolute shrinkage and selection operator)	240 subtropical lines	Shikha et al. (2017)
	300 inbred lines	González-Camacho et al. (2012)
	300 inbred lines	Crossa et al. (2010)
	300 inbred lines	Crossa et al. (2011)
MLR (Multiple linear regression)	1062 DH/F2	Lorenzana & Bernardo (2009)
RBFNN (Radial basis function neural networks)	300 inbred lines	González-Camacho et al. (2012)
RF (Random forest)	240 subtropical lines	Shikha et al. (2017a)
RR (Ridge regression)	240 subtropical lines	Shikha et al. (2017a)
BL Bayes A Bayes B Bayes C RKHS RR-BLUP	358 inbred lines and 235 DH lines	Badji et al. (2021)

Table 1.5 continued

Model	Population	References
BRR		
BLR	212 Recombinant inbred lines and 75 DH lines	Liu et al. (2018)
RR- BLUP		
G-BLUP		
Bayes A		
Bayes B		
Bayes C		
RKHS		
RR-BLUP	352 inbred lines	Heslot et al. (2012)
Bayes C		
RKHS		
Support vector machine (SVM)		
Elastic Net		
RF		
BL		

Abbreviations: RR-BLUP, ridge regression best linear unbiased prediction; G-BLUP, genomic best linear unbiased prediction; BL, Bayesian least absolute shrinkage and selection operator; RKHS, reproducing kernel Hilbert space; RF, random forest; BRR, Bayesian ridge regression; BRNN, Bayesian regularized neural networks; RBFNN, radial basis function neural networks; RR, ridge regression; MLR, multiple linear regression; BGLR, Bayesian generalized linear regression; SVM, Support vector machine

Table 1.6 Genomic models and prediction accuracies in maize using genomic selection

Study	Markers used	GS model used	Prediction accuracy	References
Genomic prediction accuracies of tropical maize resistance to fall armyworm and weevils grain weight loss	Diversity Arrays Technology (DArTs)	BL	0.79	Badji et al. (2021)
		Bayes A	0.79	
		Bayes B	0.78	
		Bayes C	0.78	
		RKHS	0.77	
		RR-BLUP	0.79	
		BRR	0.78	
		BLR	0.79	
Predicting evaluation accuracy of different models on grain yield	Single nucleotide polymorphism (SNPs)	RR- BLUP	0.38	Liu et al. (2018)
		G-BLUP	0.39	
		Bayes A	0.39	
		Bayes B	0.4	
		Bayes C	0.39	
		RKHS	0.39	
Selections for drought tolerance	SNPs	RR BL RF RKHS Bayes A, B	0.28-0.93	Shikha et al. (2017)
Selection of maize lines presenting a high resistance to *Stenocarpella maydis*	DArTs	RR-BLUP	0.25-0.59	dos Santos et al. (2016)
Assessing the prediction accuracy of different quantitative traits using low-density markers and genotyping-by-sequencing	SNPs	GBLUP	0.27-0.60, 0.25-0.40	Zhang et al. (2015)
Drought stress response	SNPS	GBLUP	0.40-0.50	Zhang et al. (2015)
Genomic prediction in maize breeding populations with genotyping-by-sequencing	SNPs	GBLUP, RKHS	0.51-0.59	Crossa et al. (2013)
Genome-enabled prediction of genetic values in maize using radial basis function neural networks	SNPs	BL, RBFNN, RKHS	0.20-0.93	González-Camacho et al. (2012)
Predicting the performance of F1 hybrids between recombinant inbred lines derived from two elite maize inbred lines	Simple sequence repeats (SSRs)	BLUP, RR- BLUP	0.63-0.96	Guo et al. (2012)
Evaluating the predictive ability of GS models on maize datasets on moisture and grain yield	SNPs	RR-BLUP	0.63-0.75	Heslot et al. (2012)
		Bayes C	0.63-0.75	
		RKHS	0.64-0.79	
		SVM	0.32-0.45	
		Elastic Net	0.61-0.75	
		RF	0.60-0.73	
		BL	0.63-0.75	
Predicting the accuracy of genotypic value for marker-based selection in bi-parental plant populations	SNPs, SSRs, Restriction fragment length polymorphism (RFLPs)	G-BLUP, MLR	0.26-0.74	Lorenzana & Bernardo (2009)

Abbreviations: RR-BLUP, ridge regression best linear unbiased prediction; G-BLUP, genomic best linear unbiased prediction; BL, Bayesian least absolute shrinkage and selection operator; RKHS, reproducing kernel Hilbert space; RF, random forest; BRR, Bayesian ridge regression; BRNN, Bayesian regularized neural networks; RBFNN, radial basis function neural networks; RR, ridge regression; MLR, multiple linear regression; BGLR, Bayesian generalized linear regression; SVM, Support vector machine.

1.6 Prediction accuracy

The accuracy of GS is affected by several factors, such as sample size, trait heritability, genetic linkage, marker density, and linkage disequilibrium (Goddard et al., 2009; Wang et al., 2018). A larger training set increases accuracy by incorporating more phenotypic and genotypic profiles (Heslot et al., 2012; Wang et al., 2018). Highly heritable traits generally have superior accuracy to low heritable traits (Hayes et al., 2009). For example, Zhao et al. (2013) achieved a prediction accuracy of 0.8 for grain moisture content in maize inbred lines with high heritability ($h^2 = 0.7$). A lower heritability ($h^2 = 0.5$) resulted in less accurate prediction (0.26) for stalk lodging in maize inbred lines (Lorenzana & Bernardo, 2009).

Prediction accuracy is affected by genetic relatedness in the training and testing sets. Accuracy is high when genetically closely related individuals are used (Hayes et al., 2009; Albrecht et al., 2011; Clark et al., 2012; Cooper et al., 2014). Average prediction accuracies for grain yield were good (0.72 to 0.74) when there was relatedness between the TP and the test group. Prediction accuracies decreased (0.47 to 0.48) for unrelated families (Albrecht et al., 2011).

Marker density affects genomic prediction accuracy. High-density markers increase accuracy (Wang et al., 2018). Crossa et al. (2010) reported that 1148 SNP markers produced a lower prediction accuracy of 0.5 for grain yield, while 5500 markers provided a higher accuracy of 0.8. Linkage disequilibrium between markers and QTL also affects prediction accuracy. The accuracy of breeding generations can be reduced without LD between the marker and the trait locus (Desta & Ortiz, 2014). Use of multiple or whole genome loci is not effective without QTL segregation. QTL isolation depends on the number of QTLs and population size. Smaller populations have fewer polymorphic loci and QTLs. Hence, recalculating marker effects every few generations improves the prediction accuracy for GS. Shengqiang et al. (2009) reported that more accurate predictions rely on marker information than QTL associations. Higher prediction accuracies are the success factor for implementing GS. Factors which affect prediction accuracy (e.g. data quality, population size and model) should be optimised when designing the GS project.

1.7 Integration with modern breeding technologies

Genomic selection enhances selection efficiency when integrated with other technologies and innovations such as genome editing, high-throughput phenotyping and genotyping, DH technology, and speed breeding (Hickey et al., 2019; Voss-Fels et al., 2019; Watson et al., 2019). The combined use of GS and genome editing in maize breeding was explored by Johnsson et al. (2019), Wallace et al. (2018) and Bernado (2017). Bernardo (2017) suggested using the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology to create targeted recombination breakpoints along chromosomes. Using this technology with GS helps increase the rate of genetic gains for quantitative traits in maize. This approach allows for applying genomic prediction to foretell marker effects, which are used to pinpoint the best recombination sites on each chromosome.

Using GS and speed breeding to select parents for future generations enhances genetic gains by reducing breeding generations (Watson et al., 2017; Hickey et al., 2019). Integrating GS with speed breeding enables the efficient selection of breeding lines within a shorter timeline (Krishnappa et al., 2021). This leads to increased genetic gains through fewer breeding cycles and reduced generation intervals. Genotyping costs are a major barrier to implementing GS in poorly resourced breeding programs. High-throughput sequencing methods, such as whole genome shotgun sequencing, can be used with GS to significantly lower genotyping costs (Crossa et al., 2013). Technow & Gerke (2017) studied GS using high-throughput genotyping and genotype imputing, which was cost-effective for genotyping large populations. The GEBV values estimated from imputed marker scores were almost the same as those estimated with true marker scores, indicating that genotyping by sequencing and genotype imputing can be an effective method of genotyping large populations when using GS.

Genomic selection can be combined with rapid disease-resistance gene identification and cloning methods (Steuernagel et al., 2017). Using GS combined with marker-assisted selection (MAS) can aid in accumulating and maintaining minor gene variants that lead to effective resistance. Also, MAS can be utilized to transfer and pyramid resistance genes. Steuernagel et al. (2017) pinpointed that such a strategy will reduce selection pressure on pathogen variations. Genomic selection and gene pyramiding can be combined to stack relevant haplotypes in desirable genetic backgrounds of maize to develop elite inbred lines. Linkage disequilibrium units are used to designate genomic sections, and optimal haplotypes are stacked using a suitable genetic design (Hickey et al., 2019; Kemper et al., 2012).

Genomic selection can be combined with high-throughput phenotyping methods to obtain accurate phenotypic information for traits of interest (White et al., 2012; Cobb et al., 2013; Watanabe et al., 2017). Jia & Jannink (2012) reported that high throughput phenotyping could predict complex traits such as grain yield in multi-trait GS modelling. Multi-trait GS modelling can improve genomic prediction accuracy, notably for correlated traits. Daetwyler et al. (2015) reported that GS could be combined effectively with doubled haploid breeding. This results in the selection of haplotypes with desirable genetic backgrounds (i.e., Optimal Haploid Value Selection), enabling the prediction of the best-doubled haploids from a segregating population. Integrating phenotypic and genomic selection with modern breeding technologies harnesses the maize breeding paradigm to release commercial cultivars with market-desired product profiles.

1.8 Conclusions

The low and stagnant yields in maize require a fast and precise breeding strategy that can reduce the yield gap by developing high yielding and climate resilient varieties. Genomic and phenotypic selection offer fast selection for hybrid breeding and commercialization. Genomic selection (GS) can speed up crop breeding by identifying desired genotypes and helping commercialize preferred varieties faster. Genomic selection is, however, not widely used in public and private breeding programs, notably in Africa. This review presented the importance, current status, challenges and opportunities for GS to accelerate genetic gain in economic traits to accelerate the breeding of high-yielding maize cultivars. The information presented will guide breeders in creating market-preferred varieties using GS and integrated technologies and innovations.

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CHAPTER TWO

Genetic Diversity and Population Structure Analyses of Tropical Maize Inbred Lines Using Single Nucleotide Polymorphism Markers

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Abstract

Analyses of the genetic distance and composition of inbred lines are a prerequisite for parental selection and to exploit heterosis in plant breeding programs. The study aimed to assess genetic diversity and population structure of a maize germplasm panel comprising 182 founder lines and 866 derived inbred lines using Single Nucleotide Polymorphism (SNP) markers to identify genetically unique lines for hybrid breeding. The founder lines were genotyped with 1201 SNPs, and the derived lines with 1484 SNPs. Moderate genetic variation, with genetic diversity ranging from 0.004 to 0.44 with a mean of 0.25, was recorded for the founder lines, while corresponding values of 0.004 to 0.34 with a mean of 0.13 were recorded for the derived lines. Heterozygosity values ranging from 0.00 to 0.24 and a mean of 0.08 were recorded for both lines. Of the SNP markers used, 82% of the 1201 markers and 84% of the 1484 markers exhibited polymorphism information content ranging from 0.25 to 0.50. Analysis of molecular variance revealed significant genetic differences ($P \leq 0.001$) among and within populations in the founder and derived lines. Most detected variations, i.e., 97% and 88.38%, were attributed to within populations in the founder and derived lines, respectively. Population structure analysis identified three distinct subpopulations among founder lines and two among derived lines. Cluster analysis supported the population structure. The following genetically distant founder and derived inbred lines were selected: G15NL337 and G15NL312 (Cluster 1), 15ARG152 and RGS-PL44 (Cluster 2), RGS-PL44 and 15ARG149 (Cluster 2), and RGS-PL33 and RGS-PL44 (Cluster 2), respectively. The selected lines are genetically distinct and recommended for marker-assisted hybrid maize breeding to exploit the frequency of beneficial alleles. This study provides valuable insights for maize breeding programs, enabling the exploitation of beneficial alleles and contributing to improved crop yields and food security through hybrid breeding.

Keywords: *cluster analysis, genetic diversity, genetic variation, heterozygosity, molecular variance, single nucleotide polymorphism markers, population structure analysis*

2.1 Introduction

Globally, maize (*Zea mays* L., 2n = 2x = 20) is the second most widely cultivated cereal crop after wheat, with an estimated production area of 204 million hectares and 1, 212 million tonnes of grain production annually (FAOSTAT, 2024). Maize plays a pivotal role in food security and global economies. It is the leading staple food in developing countries and a crucial raw material for the livestock and processing industries (Cairns et al., 2013; Erenstein et al., 2022). Maize is a strategic crop in Africa's food systems, providing approximately 30% of the continent's energy intake. In Africa, smallholder farmers produce maize and trade it in local markets for household food security and cash income.

South Africa is the leading maize producer in Africa, with a production of 14 million metric tonnes per annum, and ranks as the world's seventh largest maize exporter after Brazil, USA, Argentina, Ukraine, European Union and Romania (Geyser et al., 2024). Other important maize producers in Africa are Nigeria (11.05 million tonnes of grain per year), Ethiopia (10.2 million tonnes), and Egypt (7.2 million tonnes) (FAOSTAT, 2024). The total maize production in Africa is 91 million tonnes annually, far below the demand of approximately 150 million tonnes. The grain deficit is met through imports notably from Argentina, Ukraine and Brazil. In 2020, Argentina was the top maize exporter to Africa, accounting for a monetary value of $1.8 billion, followed by Ukraine ($856 million) and Brazil ($824 million) (FAOSTAT, 2024).

Africa accounts for only 8% of the global maize production. The mean maize grain yield in the region is low at 2.1 t/ha compared to the worldwide average of 5.8 t/ha. This yield gap is attributed to a combination of abiotic stresses (e.g., heat and drought stress, flooding, waterlogging, poor soil fertility, and soil erosion) (Leitner et al., 2020; Santpoort, 2020; Thomas, 2020; Siatwiinda et al., 2021) and biotic stresses, including plant diseases (e.g., grey leaf spot, maize streak virus, maize lethal necrosis disease, *Phaesopharia* leaf spot and northern corn leaf blight), parasitic weeds (e.g. *Striga* species) and insect pests (e.g., the fall armyworm, stem borers, cutworms, termites and leafhoppers). These stresses contribute to substantial yield losses and crop failure in the region (Cairns et al., 2013; Beyene et al., 2017; Keno et al., 2018). Therefore, genetic innovations and modern production technologies are crucial to improving the yield potential and closing the yield gap.

Hybrid maize breeding is critical in developing resilient, locally adapted, and high-performing cultivars. Hybrids achieve increased yields, withstand diseases and pests more effectively, and provide better nutritional content. Furthermore, they demonstrate exceptional resilience to drought, heat, flooding, and poor soil quality (Varshney et al., 2021). The success of hybrid breeding depends on heterosis, or hybrid vigour, which is maximized by selecting genetically distant and contrasting inbred lines (Swarup et al., 2021).

Genetic variation allows for the selection of favourable genetic combinations among different and complementary parents (Melani and Carena, 2005; Lu et al., 2009; Zhou et al., 2009; Prasanna, 2012). A well-characterized genetic resource is essential for identifying potential parents, heterotic groups, and guiding conservation (Reif et al., 2004; Legesse et al., 2006; Giordani et al., 2019). Analyses of genetic structure and diversity provide valuable insights into the relationships between breeding lines, which can guide hybrid breeding (Buckler et al., 2006; Adu et al., 2019). Several studies have assessed the population structure and genetic diversity among varied maize populations and contrasting test environments and marker systems. For instance, Lu et al., (2009) analyzed 770 maize lines, identifying distinct population structures and genetic divergence between temperate and subtropical/tropical germplasm using 1,034 SNPs. Yan et al., (2009) reported well-delineated genetic structures between temperate and tropical lines among 632 inbred lines. Adu et al., (2019) assessed genetic diversity in 94 tropical maize inbred lines, clustering them by pedigree, selection history, and endosperm color. Furthermore, Wen et al., (2011) examined 359 maize inbred lines developed by CIMMYT and IITA, displaying variable tolerance to abiotic and biotic stresses. The present and past findings indicate the need for rigorous genetic diversity analysis of candidate test populations using high throughput SNP markers to appraise the genetic structure and lineage and guide selection and breeding. Notably, hybrids developed from diverse heterotic groups consistently outperform their parents in grain yield and yield components traits (Talabi et al., 2017).

Elite inbred lines can be assigned to distinct heterotic groups using phenotyping, pedigree analyses, and genetic distance estimates (Adu et al., 2019). Morphological, biochemical, and molecular markers are commonly used in genetic diversity analysis and genetic grouping (Govindaraj et al., 2015). DNA markers (e.g., Single Nucleotide Polymorphisms (SNPs), Simple Sequence Repeats (SSRs), Restriction Fragment Length Polymorphisms (RFLPs), Randomly Amplified Polymorphic DNAs (RAPDs), and Amplified Fragment Length Polymorphisms (AFLPs)) have become complementary tools to phenotyping tools.

Genetic markers have high repeatability with limited influence from genotype x environment interaction effects. SNPs have become the preferred choice of markers to genotype maize populations due to their low cost per data point, widespread presence in the genome, specific location at genetic loci, co-dominance, amenable for high-throughput analysis, and lower rates of genotyping errors (Mammadov et al., 2012; Semagn et al., 2012). SNPs have been used to identify distinct subpopulations (Van Inghelandt et al., 2010), determine genetic diversity within and between landraces (Arca et al., 2021), assess genetic diversity of early maturing white and yellow tropical maize inbred lines (Adu et al., 2019), determine the rate of decay of linkage disequilibrium (Lu et al., 2011), and discern population structure (Zhang et al., 2016).

Analyses of the genetic distance and composition of inbred lines are a prerequisite for parental selection and to exploit heterosis in hybrid breeding programs. Seed Co Ltd systematically bred and selected founder and derived elite maize inbred lines from two major heterotic groups to develop high-performing commercial single cross and three-way hybrids. However, there is lack of information on the genetic diversity and relationship of these lines to guide the regional maize breeding program. In this regard, the test lines should be characterized with diagnostics SNP markers to select genetically distinct and complimentary lines for marker-assisted hybrid maize breeding to exploit the frequency of beneficial alleles. Therefore, this study aimed to assess the genetic diversity and population structure of 182 founder lines and 866 derived inbred lines of maize using SNP markers to identify genetically complementary lines for hybrid breeding.

2.2 Materials and methods

2.2.1 Plant material

The study used 182 elite founder-inbred lines and 866 derived inbred lines of maize from tropical and subtropical genetic lineages. The founder lines, sourced from the Seed Co Ltd maize germplasm pool, are elite parental lines selected to develop improved maize varieties. These lines are widely utilized in breeding programs and are prominent in most released hybrid varieties in Zimbabwe. The 866 tropical inbred lines were developed using pedigree selection from diverse source populations created by crossing founder lines, selected for their adaptability to tropical and subtropical environments in sub-Saharan Africa (SSA) after rigorous testing. The lines were selected based on desirable agronomic characteristics, such as high yield potential, drought tolerance, and disease resistance. Table 2.1 summarises the list of lines and their respective heterotic groups [N3 (group 1) and SC (group 2)]. Briefly, the N3 heterotic group were originated from the Salisbury white landrace, which was cultivated in Salisbury (now known as Harare) before the introduction of hybrid maize in 1960. On the other hand, the SC group was obtained from a landrace grown on Mr Southey's farm and was named "Southern Cross". The N3 was specifically designated as "Northern Cross" to highlight the contrast with the SC inbreds.

Table 2.1 List of the founder and derived lines used in the study

Inbred lines	**Code or designation**	**Description**
Founder lines	RGS-PL65, RGS-PL08, RGS-PL09, RGS-PL68, RGS-PL63, RGS-PL62, RGS-PL32, RGS-PL38, RGS-PL53, RGS-PL20, RGS-PL10, RGS-PL58, RGS-PL06, 15ARG119, 15ARG142, 15ARG143, 15ARG148, 15ARG149, 15ARG151, 15ARG175, RGS-PL48, RGS-PL52, 15ARG104, 15ARG176, RGS-PL54, RGS-PL19, RGS-PL17, 15ARG112, 15ARG127, 15ARG129, 15ARG152, RGS-PL60, RGS-PL13, RGS-PL07, 15ARG111, 15ARG114, 15ARG132, 15ARG140, 15ARG161, RGS-PL66, RGS-PL11, RGS-PL36, RGS-PL01, RGS-PL44, 15ARG110, 15ARG123, 15ARG164, RGS-PL43, RGS-PL47, RGS-PL69, 15ARG117, 15ARG128, 15ARG158, 15ARG160, 15ARG173, RGS-PL50, RGS-PL03, RGS-PL59, 15ARG131, 15ARG137, 15ARG147, 15ARG154, 15ARG155, 15ARG159, 15ARG165, 15ARG167, 15ARG174, RGS-PL18, RGS-PL24, 15ARG106, 15ARG107, 15ARG116, 15ARG125, 15ARG126, 15ARG130, 15ARG144, 15ARG153, 15ARG171, RGS-PL05, RGS-PL45, 15ARG121, 15ARG124, 15ARG157, 15ARG168, RGS-PL64, RGS-PL56, RGS-PL55, RGS-PL15, RGS-PL21, RGS-PL02, 15ARG145, 15ARG163, 15ARG172, 15ARG109, 15ARG120, 15ARG122, 15ARG136, 15ARG138, 15ARG166, 15ARG177, RGS-PL14, RGS-PL16, RGS-PL40, 15ARG113, 15ARG115 15ARG150 15ARG156 RGS-PL57 RGS-PL61 RGS-PL49 RGS-PL37 RGS-PL12, RGS-PL22, RGS-PL0, RGS-PL39, 15ARG105, 15ARG133, 15ARG135, 15ARG139, 15ARG169, RGS-PL70, RGS-PL42, 15ARG103, 15ARG146, 15ARG170, RGS-PL67, RGS-PL41, 15ARG108, 15ARG118, 15ARG134, 15ARG141, 15ARG162	Heterotic group 1 (N3)
	16ARG16793, 16ARG16799, 16ARG16808, RGS-PL46, RGS-PL28, 16ARG16812, 16ARG16786, 16ARG16788, 16ARG16791, 16ARG16792, 16ARG16804, 16ARG16816, RGS-PL35, RGS-PL30, 16ARG16798, 16ARG16815, 16ARG16782, 16ARG16814, RGS-PL71, 16ARG16784, 16ARG16795, RGS-PL25, RGS-PL31, RGS-PL29, 16ARG16794, 16ARG180, 16ARG16790, 16ARG16802, 16ARG16806, 16ARG16811, RGS-PL33, 16ARG178, 16ARG16803, 16ARG16807, RGS-PL27, 16ARG16781, 16ARG16783, RGS-PL34, 16ARG16789, 16ARG16796, 16ARG16797, 16ARG16801, 16ARG16805, 16ARG181, 16ARG16800, 16ARG179, 16ARG16787, 16ARG16810, 16ARG16813, 16ARG16785, 16ARG16809, 16ARG1681, RGS-PL26	Heterotic group 2 – (SC)
Derived lines	G15NL01 - G15NL12; G15NL14 - G15NL20; G15NL22 - G15NL27; G15NL29 - G15NL36; G15NL281; G15NL283 - G15NL314; G15NL316 - G15NL321; G15NL323 - G15NL334; G15NL336 - G15NL342; G15NL344 - G15NL357; G15NL359 - G15NL365; G15NL367 - G15NL370, G16NL37 - G16NL50; G16NL54 - G16NL68; G16NL70 - G16NL74; G16NL77 - G16NL83; G16NL85 - G16NL99; G16NL100 - G16NL109; G16NL112 - G16NL138; G16NL141 - G16NL145; G16NL147 - G16NL153; G16NL155, G16NL156, GL17NL157 - G17NL170; G17NL172 - G17NL180; G17NL182 , G17NL226; G17NL228 - G17NL239; G17NL241 - G17NL242, G18NL206, G18NL240 - G18NL246; G18NL248 - G18NL254; G18NL257 - G18NL267; G18NL269 - G18NL271; G18NL273 - G18NL280	Heterotic group 1 (N3)
	G16NL677 - G16NL813; G16NL815 - GL16NL850; GL16NL852 - GL16NL921 G17NL373 - G17NL467; G17NL469 - G17NL484; G17NL486 - G17NL664 G18NL665 - G18NL676	Heterotic group 2 (SC)

2.2.2 Genotyping of inbred lines

2.2.2.1 Sample collection

The lines were field established at Rattray Arnold Research Station (RARS) in Zimbabwe. RARS is situated at Longitude 31°12′ 41.35″ E, Latitude 17°40′ 20.07″ S, at an altitude of 1360 metres above sea level (Figure 2.1). The climate is subtropical, with average monthly temperatures ranging from 28 to 32°C between November and April. The total annual precipitation received at RARS is 865mm, mostly between November and April. RARS is located in the mid-altitude moist environments, which are the primary maize-growing areas in Southern Africa. Ten kernel samples were collected for each line. The samples were placed in envelopes, which were sealed and accurately labelled. Healthy and disease-free kernels were sampled for genotyping.

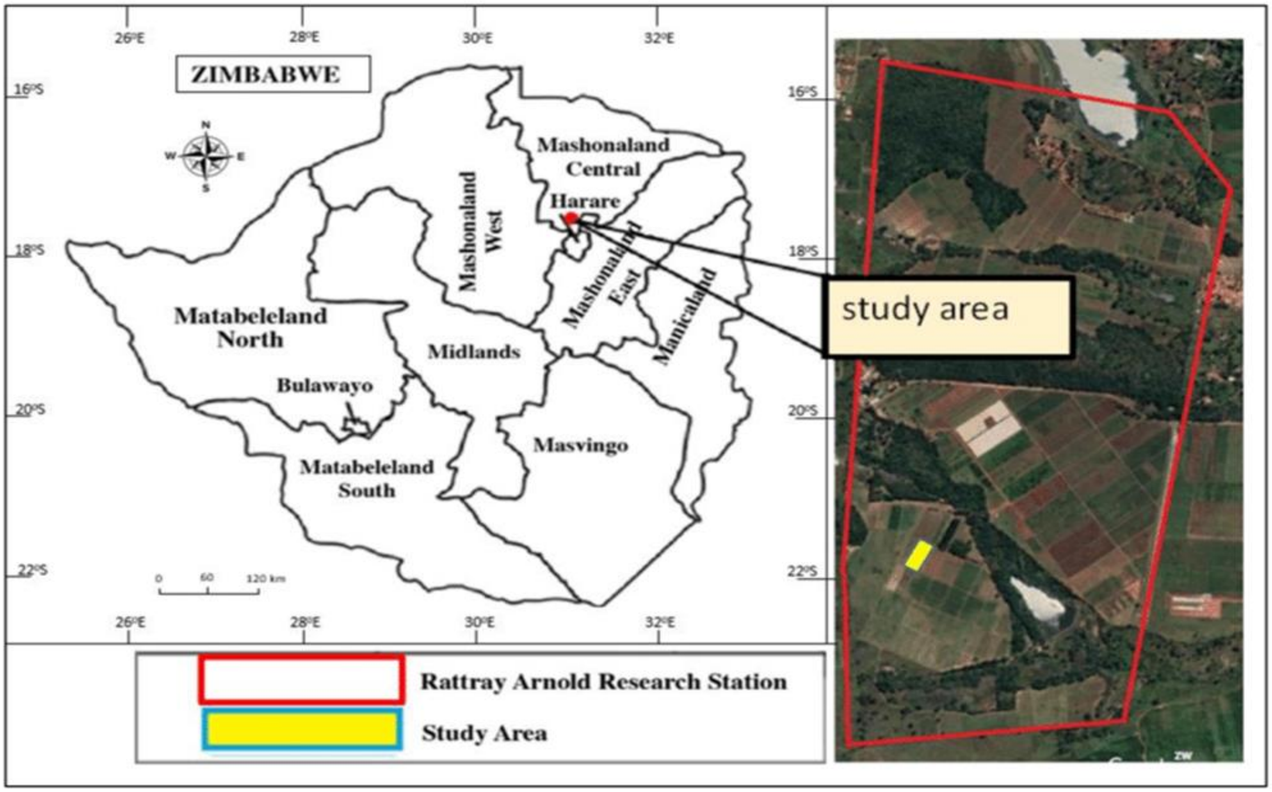

Figure 2.1 Map of Zimbabwe displaying the on-station study site, Rattray Arnold Research Station.

2.2.2.2 DNA extraction

DNA extraction and SNP genotyping were performed in the Limagrain Laboratory in France following established protocols. Genomic DNA was isolated from maize kernels using the Kompetitive Allele-Specific (KASP) custom method, and genotyping was facilitated by the LGC KASP system (accessible at http://www.lgcgenomics.com). DNA extraction was done from ten kernels per inbred line, with positive controls included in the initial six wells of a 96-well plate. DNA purity and concentration were assessed using a Nanodrop device (Nano Vue Plus), and the DNA was diluted to concentrations ranging from 81 to 188 µg/L. The DNA was then arrayed onto 384 PCR plates and genotyped using 1201 and 1484 SNP KASP markers for the 182 founder lines and 866 derived tropical lines, respectively, covering all ten maize chromosomes. Polymerase Chain Reaction amplification was performed using Kompetitive Allele-Specific PCR (KASP) primers, generating sufficient DNA for genotyping. The KASP system facilitated the genotyping process, with scanning conducted using Pheraster SNP software and processing carried out using KlusterCaller software (http://www.lgcgenomics.com).

2.2.3 Data Analysis

2.2.3.1 Analysis of molecular variance

Analysis of molecular variance (AMOVA) was conducted using GenAlEx 6.51b2 software, following the protocol outlined by Excoffier et al. (1992) to partition genetic variations among and within populations attributable to SNPs.

2.2.3.2 Characterization of SNP markers and inbred lines

SNP markers with poor amplification, uncertain allele identification, or excessive missing data (>10%) were excluded, as were markers with a minor allele frequency of less than 5%. Inbred lines with the associated data missing above 10% were removed from the analysis. After filtering, 1201 SNP markers (Table S 2.1) were used to genotype the 182 founder lines and 1484 markers (Table S 2.2), were used to genotype the 866 derived lines. The SNP markers used varied due to the use of different platforms for genotyping [Affymetrix (founder lines) and MIDSEQ (derived lines)]. The inbred lines were genotyped using KlusterCaller software (http://www.lgcgenomics.com) to detect single nucleotide polymorphisms (SNPs) and insertions/deletions (indels), enabling a thorough evaluation of genetic variation. The DARwin6 software (Perrier and Jacquemond, 2006) was used to identify unique clustering patterns and structure, and generated dendrograms and phylogenetic trees to visualize the relationships between populations.

The 1201 and 1484 markers selected were distributed among the 10 maize chromosomes for the 182 founders and 866 derived lines, respectively. The number of markers per chromosome varied from 59 to 215 in the founder lines and 76 to 231 in the derived lines. The markers were spaced at regular intervals along each chromosome, based on genetic distance expressed in centimorgan (cM). This arrangement ensured full coverage and prevented the clustering of markers, giving a complete overview of the entire genome (Table 2.2).

Table 2.2 Positioning of the 1201 and 1484 SNP markers on the maize chromosomes using linkage analysis.

Chromosome	Number of markers	Position range (Centimorgans)	Chromosome	Number of markers	Position range (Centimorgans)
1	212	16.06 - 617.22	1	231	(-29.91) - 619.29
2	114	1.21 - 443.73	2	173	(-29.69) - 444.94
3	114	(-5.18) - 520.78	3	189	(-5.00) - 521.05
4	84	2.82 - 378.74	4	151	(-16.32) - 385.80
5	165	5.23 - 403.51	5	141	(-2.89) - 413.67)
6	105	(-4.28) - 373.85	6	114	(-13.85) - 376.33
7	70	(-44.27) - 425.09	7	135	(-57.04) - 425.09
8	215	(-31.87) - 382.75	8	141	(-34.41) - 362.26
9	59	6.44 - 350.25	9	130	(-17.33) - 355.45
10	63	1.30 - 232.45	10	76	(-13.50) - 242.23
	1201			1484	

Genetic parameters were computed, including major allele frequency (MAF), gene diversity (He), polymorphic information content (PIC) and heterozygosity (Ho) using Power-Marker (version 3.2.5) statistical software (Liu and Muse, 2005). GD represents the likelihood of two randomly chosen individuals being different at a specific locus, measuring expected heterozygosity (Ellegren and Galtier, 2016). Gene diversity (He) was calculated as follows (Botstein et al., 1980):

$$\text{He} = 1 - \sum_{u=1}^{k} \tilde{P}^{2}{}_{u}$$

where

k= number of alleles,

$\tilde{P}^2{}_u$= frequency of the marker allele.

Polymorphic information content (PIC) values estimate a marker's discriminating power by considering the number of alleles. The formula for calculating the PIC was as follows (Botstein et al., 1980):

$$\mathrm{PIC} = 1 - \sum_{u=1}^{k} \tilde{P}^2{}_u - \sum_{u=1}^{k-1} \sum_{v=u+1}^{k} 2\tilde{P}^2{}_u \tilde{P}^2{}_V$$

where:

$\tilde{P}^2{}_u$= frequency of the marker allele,

k= number of alleles,

$\tilde{P}^2{}_u$= frequency of the marker allele,

$P^2{}_u$= frequency of the u^{th} marker,

$\tilde{P}^2{}_V$= frequency of the v^{th} marker.

The PIC values were categorized as highly informative (PIC value of the marker >0.50), (ii), moderately informative (0.25 to 0.50) or slightly informative (<0.25) (Botstein et al., 1980).

2.2.3.3 Marker call rate

The marker call rate was calculated by determining the proportion of successful genotyping calls for each marker across all samples.

Marker call rate = (Number of successful genotyping calls / Total number of samples) x 100

Where:

- Number of successful genotyping calls = the number of samples for which a genotype was successfully called for a given marker.
- Total number of samples = the total number of samples genotyped for a given marker.

2.2.4 Population structure analysis

Genetic data from 1201 and 1484 SNP markers were analyzed using the admixture model-based clustering method in STRUCTURE 2.3.4 software (Earl and vonHoldt, 2012) to infer the population structure of 182 founder lines and 866 derived lines, respectively. The analysis settings included a burn-in period of 20,000 iterations, a Markov chain Monte Carlo (MCMC) simulation length of 100,000, and six independent runs for each K value (ranging from 1 to 7) for the 182 founder lines and ten independent runs for each K value (ranging from 1 to 11) for the 866 derived lines. The optimal number of populations (K) was estimated using the Evanno et al. (2005) method, as implemented in the online STRUCTURE Harvester tool (Earl and vonHoldt, 2012).

2.2.5 Cluster analysis

The genetic relationships among the inbred lines were discerned using DARwin software (Perrier and jacquemond, 2006). The neighbor-joining method (NJ) (Saitou and Nei, 1987) was used to construct phylogenetic trees with 500 bootstraps using the 1201 SNP marker data for the 182 founder lines and 1484 SNP marker data for the 866 derived lines. Continuous dissimilarity indices were generated using the standard Euclidean similarity test using the formula described by Jacquemoud-Collet (2006), enabling the construction of dendrograms that illustrate the genetic distances between the lines:

$$d_{ij} = \sqrt{\sum_{1}^{k} \left(X_{ij} - X_{jk}\right)^{2}}$$

Where; d_{ji} is the similarity between units i and j; Xij, X_{jk} are values for variable k for units I and j, X represents the global mean, and k indicates the number of variables. The output from DARwin was imported into FigTree version 1.4.3 software (Rambaut 2016) to construct the final phylogenetic trees.

2.3 Results

2.3.1 Summary statistics for the 182 founder and 866 derived lines

Table 2.3 presents the genetic diversity parameters based on SNP markers for the 182 founder and 866 derived lines. The observed heterozygosity for the 182 founder lines was 0.08, ranging from 0.01 to 0.24. Gene diversity ranged from 0.00 to 0.44, with a mean of 0.25, indicating a moderate level of genetic variation. The mean major allele frequencies were 0.85, ranging from 0.50 to 0.99. The polymorphic information content (PIC) ranged from 0.00 to 0.50, with a mean of 0.37. Most markers (82%) were moderately informative (PIC ≥ 0.25), while 18% were slightly informative (PIC < 0.25) (Figure 2.2). Minor allele frequencies ranged from 0.10 to 0.50, with a mean of 0.28. The marker call rate was high, with a mean of 89.99%, varying from 85.00% to 95.00%.

The major allele frequencies based on the 1,484 SNP markers ranged from 0.50 to 0.99, with a mean of 0.82. Gene diversity ranged from 0.00 to 0.34, with a mean of 0.25, while observed heterozygosity averaged 0.08, ranging from 0.00 to 0.21. The PIC values ranged from 0.00 to 0.50, with a mean of 0.41. Most markers (84%) were moderately informative (PIC ≥ 0.25), while 16% were slightly informative (PIC < 0.25) (Figure 2.2). The minor allele frequencies ranged from 0.08 to 0.50, with a mean of 0.30. The marker call rate ranged from 37.22% to 100%, with a mean of 96.64%, indicating a high genotyping success rate.

Table 2.3 Genetic diversity parameters for 182 founder and 866 derived inbred lines of maize calculated using 1201 and 1484 SNP markers, respectively

Statistics/genetic parameters	MaF	He	Ho	PIC	MAF	Call Rate
			Founder lines			
Minimum	0.50	0.00	0.00	0.00	0.10	85.00
Maximum	0.99	0.44	0.24	0.50	0.50	95.00
Mean	0.85	0.25	0.08	0.37	0.28	89.99
			Derived lines			
Minimum	0.50	0.00	0.00	0.00	0.08	37.22
Maximum	0.99	0.34	0.21	0.50	0.50	100.00
Mean	0.82	0.13	0.08	0.41	0.30	96.64

MaF=Major allele frequency, He=Gene diversity, Ho=Heterozygosity, PIC=Polymorphism information content, MAF=Minor allele frequency.

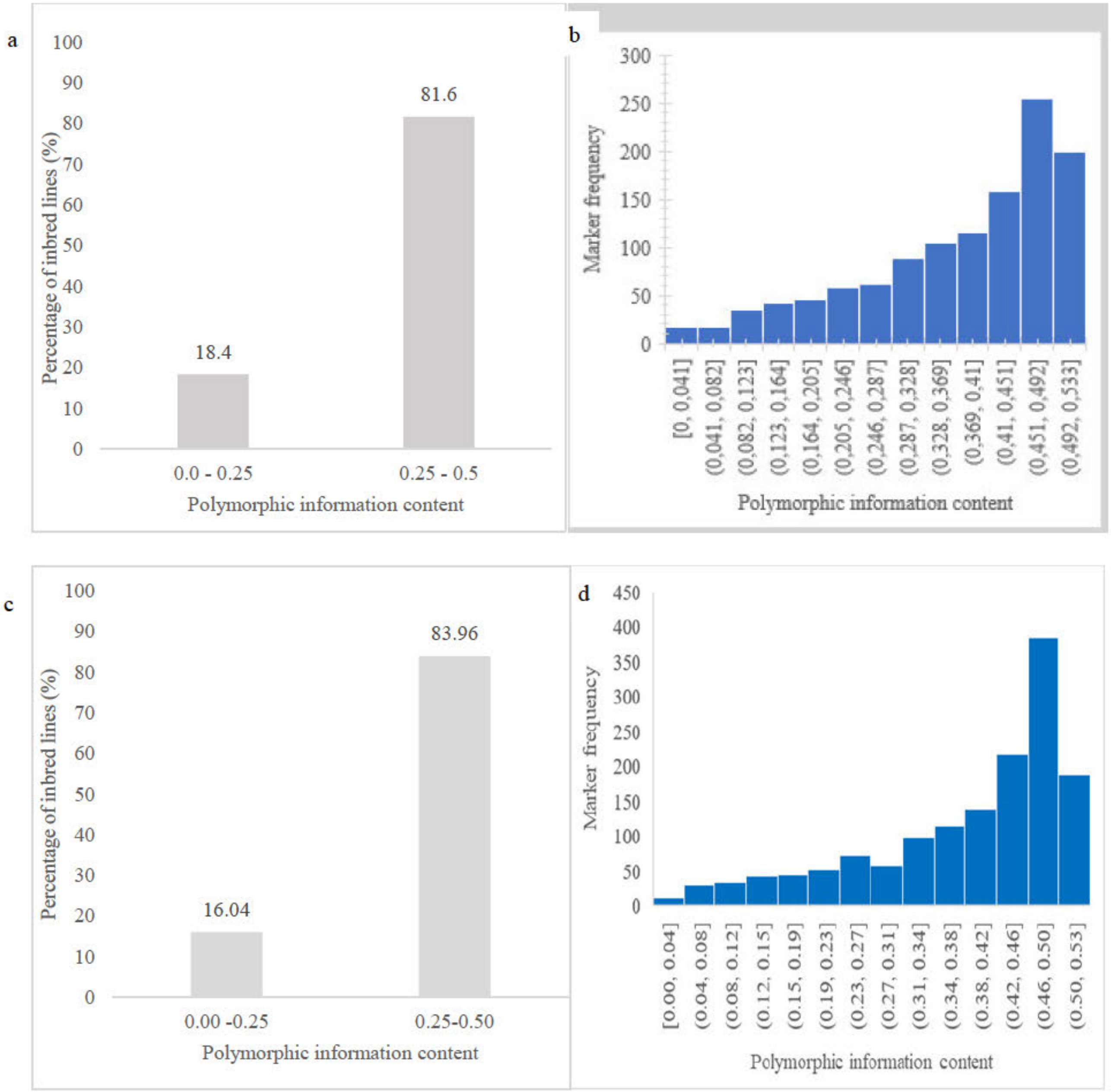

Figure 2.2 Frequency distribution and marker frequency of 182 parental inbred lines (a and b) and 866 derived inbred lines (c and d) calculated using 1201 and 1484 SNP markers, respectively, based on polymorphic information content.

2.3.2 Analysis of molecular variance

Analysis of molecular variance (AMOVA) revealed significant genetic differences ($P \leq 0.001$) among and within populations (Table 2.4). Most genetic variation was attributed to within-population variation, accounting for 97% and 88.38% of the total variation in the founder and derived lines, respectively (Table 2.4).

Table 2.4 Summary of analysis of molecular variance comparing among and within maize populations of 182 founder inbred lines and 866 derived inbred lines based on 1201 and 1484 SNP markers, respectively

Founder inbred lines					
Source of variation	Degrees of Freedom	Sum of Squares	Estimated Variance	Percentage of Variation	F Statistics
Among Pops	1	344.555	6.062	3	≤ 0.001
Within Pops	180	39464.951	219.25	97	≤ 0.001
Total	181	39809.505	225.312	100	

Derived inbred lines					
Source of variation	Degrees of Freedom	Sum of Squares	Estimated Variance	Percentage of Variation	F Statistics
Among Pops	2	6568.76	22.98	11.62	≤ 0.001
Within Pops	864	161234.52	179.84	88.38	≤ 0.001
Total	866	167234.52	202.82	100	

Pops=populations

2.3.3 Population structure of the germplasm panel

The population structure analysis demarcated the 182 maize lines into three distinct subpopulations (Figure 2.3), with ΔK peaking at K=3. The tripartite division captured the underlying genetic diversity, with each cluster varying in size and composition. Subpopulation 1 consisted of 38 lines, Subpopulation 2 had 100 lines, and Subpopulation 3 had 44 lines (Supplementary Table 2.4). Further, the 866 maize lines were partitioned into two subpopulations (Figure 2.4), with the highest ΔK value at K=2, indicating a bipartite division. The two clusters, identified by the highest median log-probability values (Ln(Pr(Data))), differed in size and composition, with cluster 1 composed of 328 lines and cluster 2 having 538 lines (Table S 2.5).

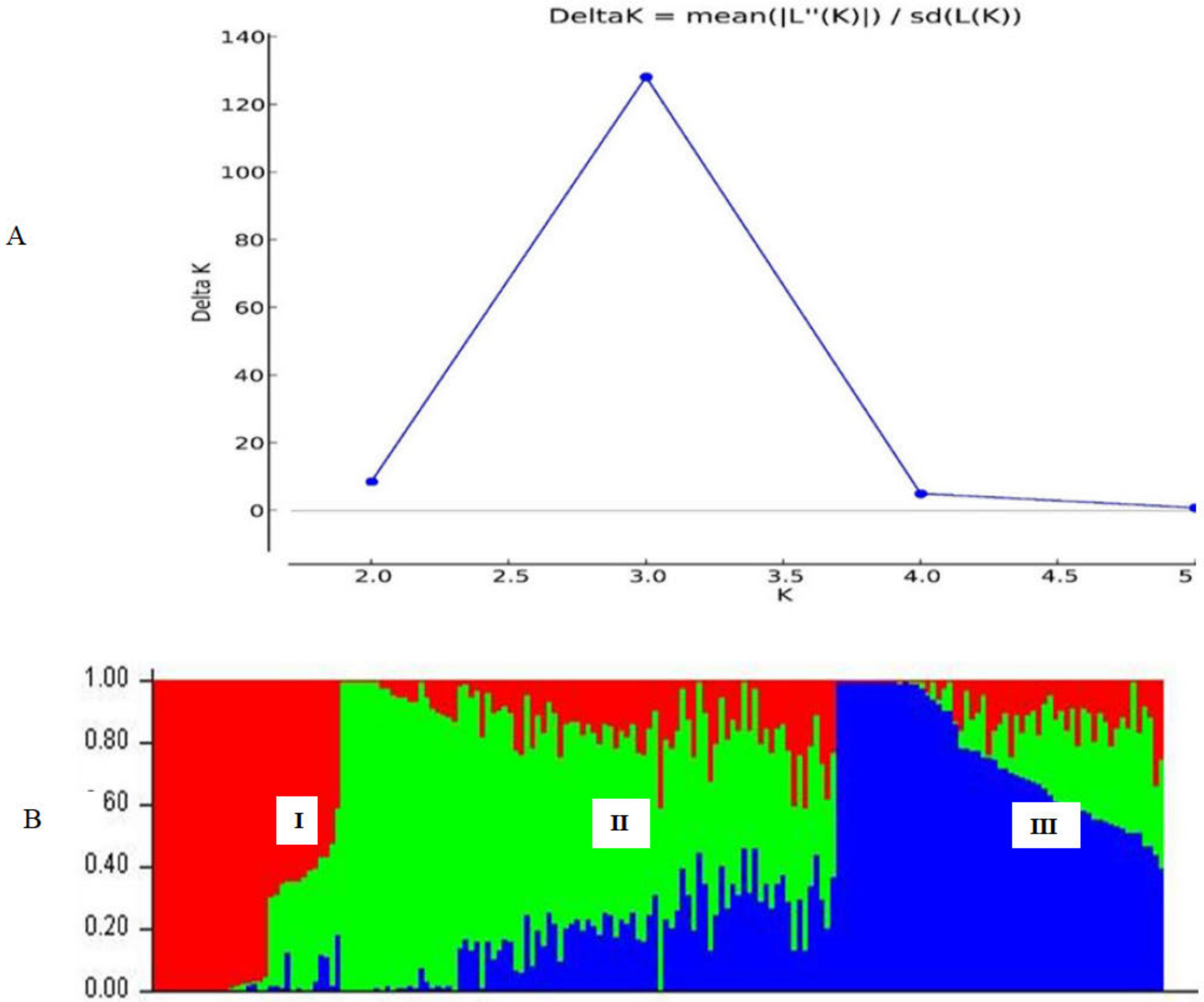

Figure 2.3 Three sub-populations discerned for the 182 founder inbred lines of maize genotyped using 1201 SNP markers. A - Best Delta K estimation via the Evanno method. B - Estimated population structure of 182 maize inbred lines revealed by 1201 SNP markers for K=3. Where, I = Sub-population 1, II = Sub-population 2, III = Sub-population 3.

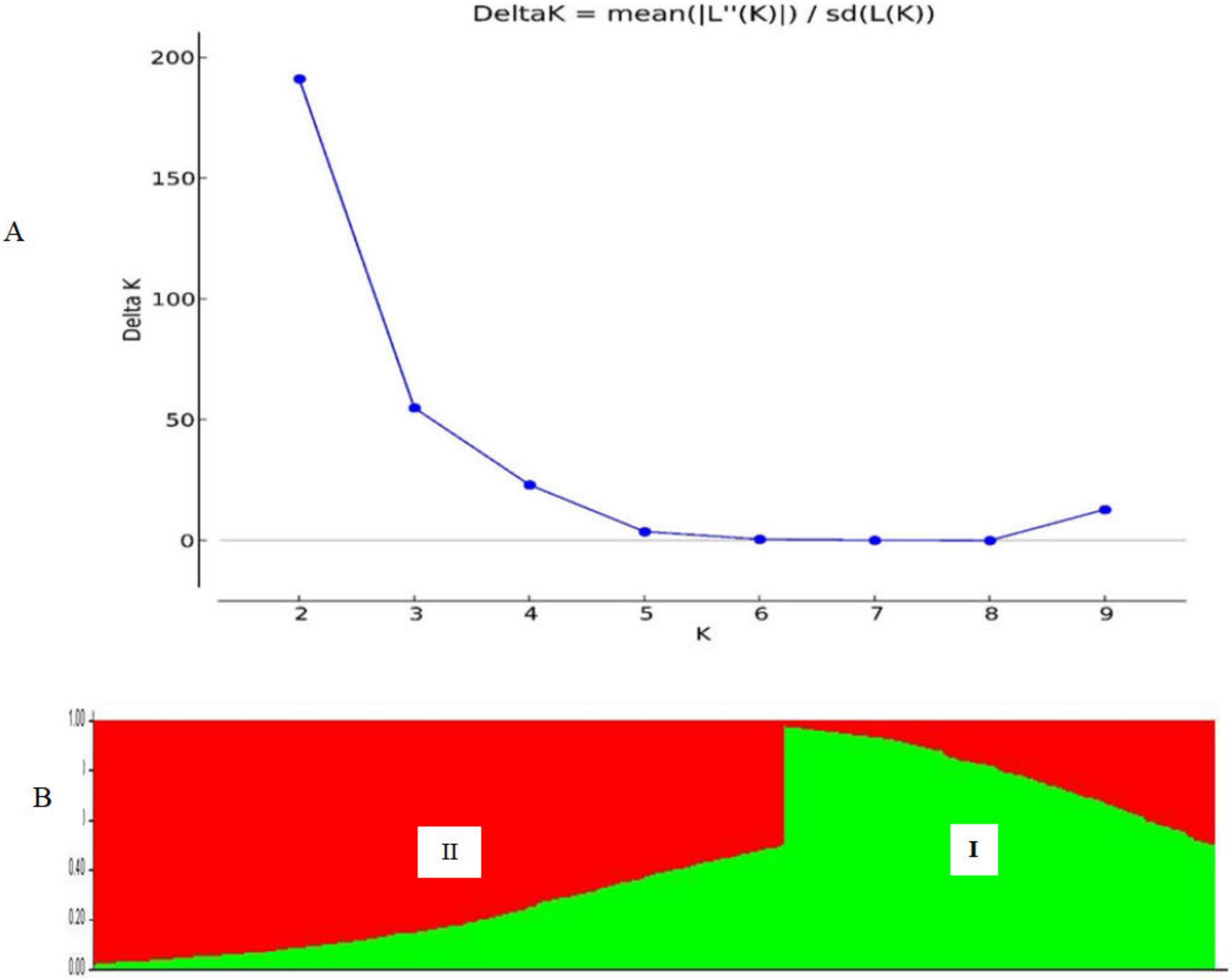

Figure 2.4 Two sub-populations resolved among the 866 derived inbred lines of maize using 1484 SNP markers. A - Best Delta K estimation via the Evanno method. B - Estimated population structure of 866 maize inbred lines revealed by 1201 SNP markers for K=2. I= sub-population 1, II= Sub-population 2.

2.3.4 Cluster analysis

2.3.4.1 Genetic distance and cluster analysis of 182 founder inbred lines

Genetic distance estimates based on SNP markers among the 182 maize inbred lines revealed variable genetic diversity ranging from 0.006 (16AG16785 vs 16AG16786) to 0.435 (RGS-PL33 vs RGS-PL44) (Table S 2.6). The mean genetic distance for all pairwise comparisons was 0.25, indicating moderate genetic diversity among the lines. Low genetic distances were detected between several pairs of inbred lines, including 16AG16786 and 16AG16785 (0.006), 16AG16801 and 16AG16802 (0.013), and RGS-PL17 and RGS-PL55 (0.021), suggesting a high degree of genetic similarity between these lines. In contrast, high genetic distances were estimated between RGS-PL33 and RGS-PL44 (0.435), 15AG152 and RGS-PL44 (0.432), indicating a more distant genetic relationship.

Genetic grouping based on population structure analysis confirmed the results of the cluster diagram that resolved the 182 genotyped inbred lines into three major clusters (Figure 2.5). Each cluster was partitioned into sub-clusters, with Cluster II being the largest (comprising 55% of parental inbred lines), followed by Cluster III (24% of inbred lines), and Cluster I (21% of inbred lines). These clusters corresponded to sub-populations 1 (red), 2 (green), and 3 (blue) from the structure analysis, respectively. The phylogenetic tree provided a visual representation of the genetic relationships among the inbred lines, supporting the findings of the population structure analysis.

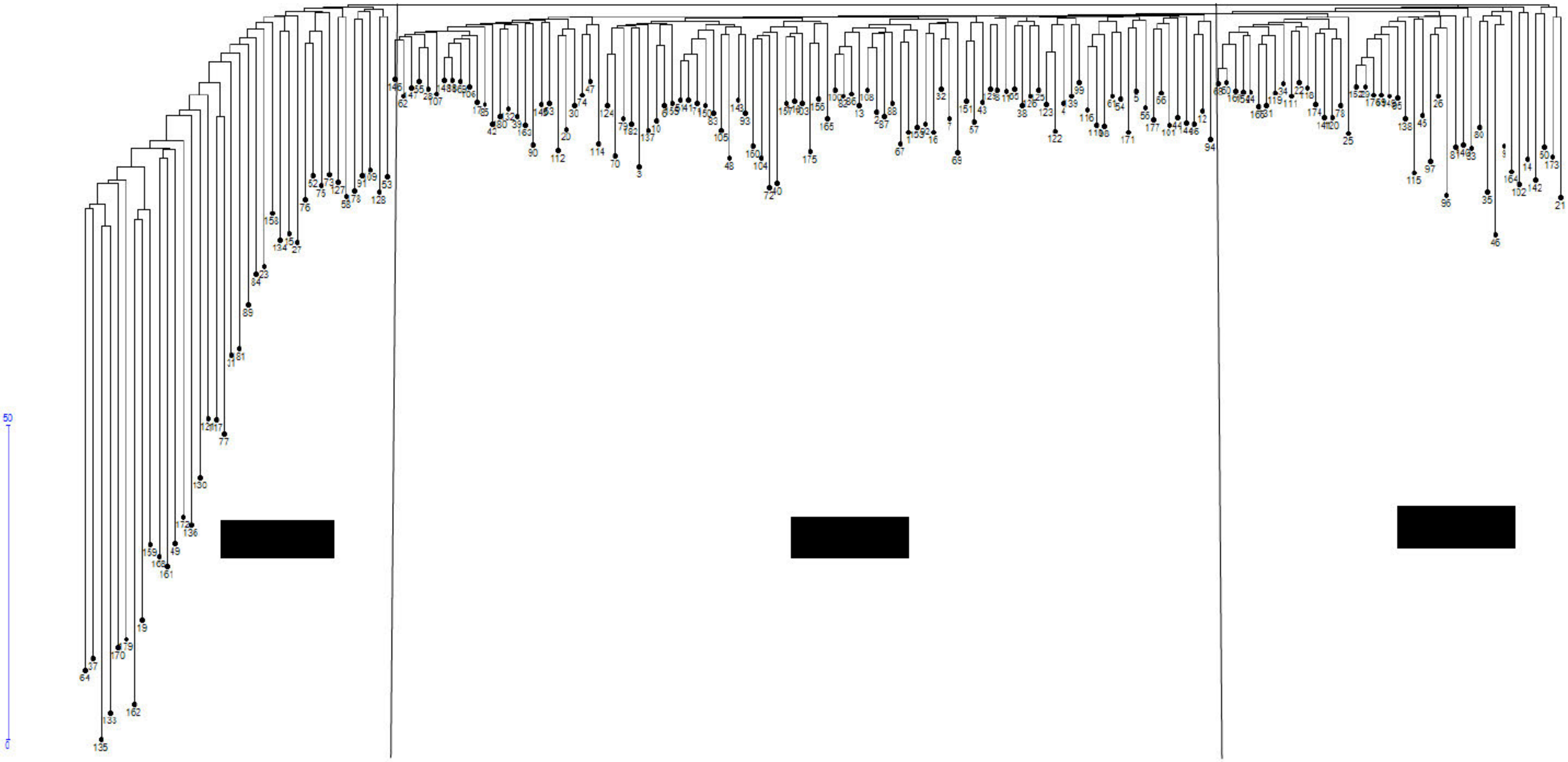

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Figure 2.5 Cluster diagram showing the relationships between 182 inbred lines based on 1201 SNP markers. See Table S 2.3 for codes of genotypes.

2.3.4.2 Genetic distances and cluster analysis of 866 derived inbred lines

The genetic distance between pairwise comparisons based on 1484 SNP markers for the 866 derived lines ranged from 0.004 to 0.336 (Table S 2.7), with a mean genetic distance of 0.14. Most lines (77%) had genetic distances ranging from 0.004 to 0.20, while 33% had distances above 0.20, ranging from 0.20 to 0.34 (Figure 2.6). The lowest genetic distance (0.004) was recorded between the inbred lines G17NL211 and G17NL210. Other pairs of lines with low genetic distances included G17NL473 and G17NL472 (0.005), G17NL194 and G17NL472 (0.008), G16NL854 and G16NL857 (0.009), G17NL602 and G17NL603 (0.011), and G16NL919 and G16NL920 (0.015). Conversely, the highest genetic distance was recorded between lines G15NL337 and G15NL312 (0.336), followed by G15NL349 and G15NL310 (0.31), G15NL303 and G15NL357 (0.303), G15NL327 and G15NL353 (0.301), G15NL292 and G15NL284 (0.299), and G15NL355 and G15NL301 (0.298). Cluster analysis of the 866 derived lines based on SNP marker genetic distance estimates grouped the lines into two distinct clusters (Figure 2.7), agreeing with the structure analysis (Figure 2.4). Each cluster was partitioned into sub-clusters. Cluster I, corresponding to Sub-population 1 from the population structure analysis (highlighted green), was designated as heterotic group 1 and composed 328 lines (37.88%). Cluster II, corresponding to Sub-population 2 (highlighted red), was designated heterotic group 2 with 538 lines (62.12%).

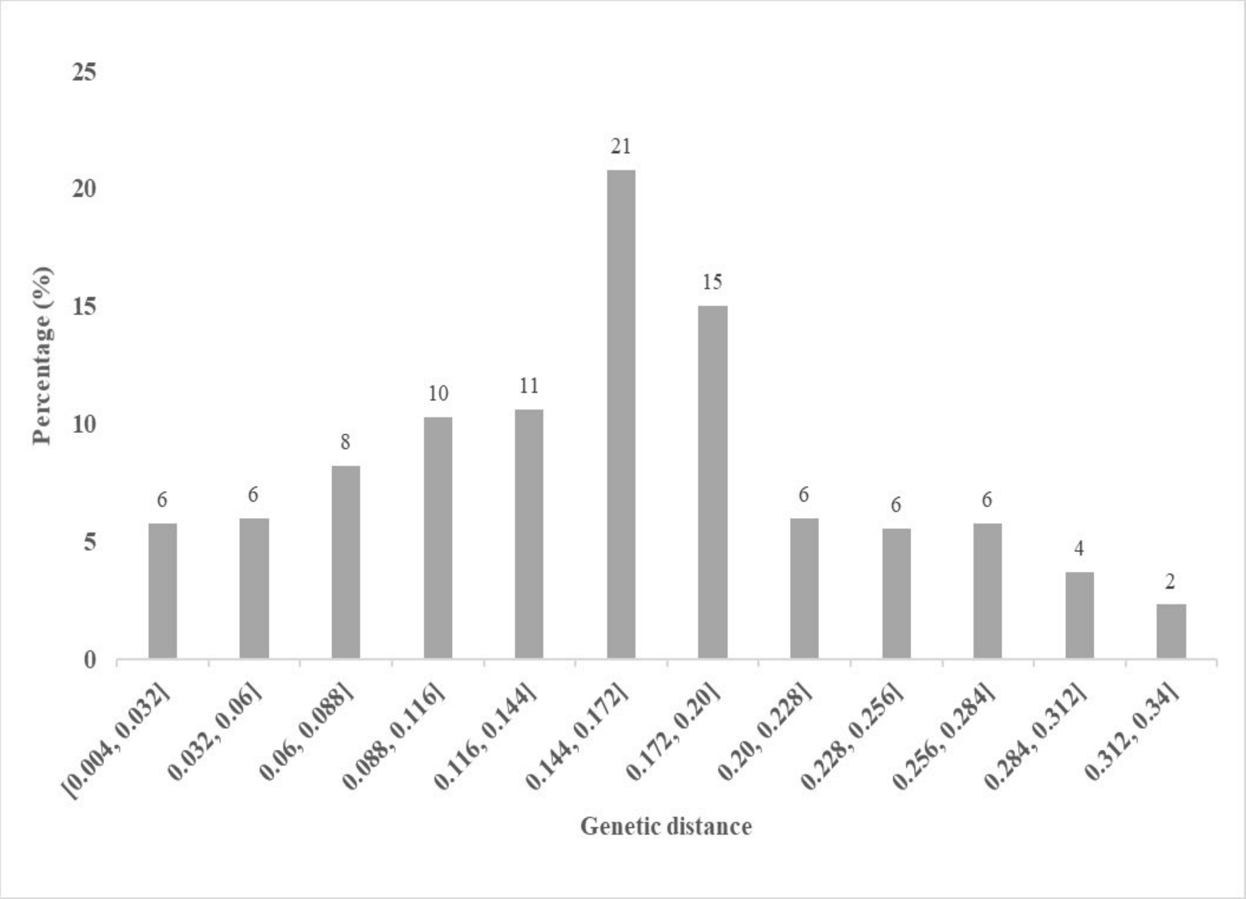

Figure 2.6 The distribution of pairwise genetic distance calculated for 866 maize inbred lines genotyped with 1484 SNPs.

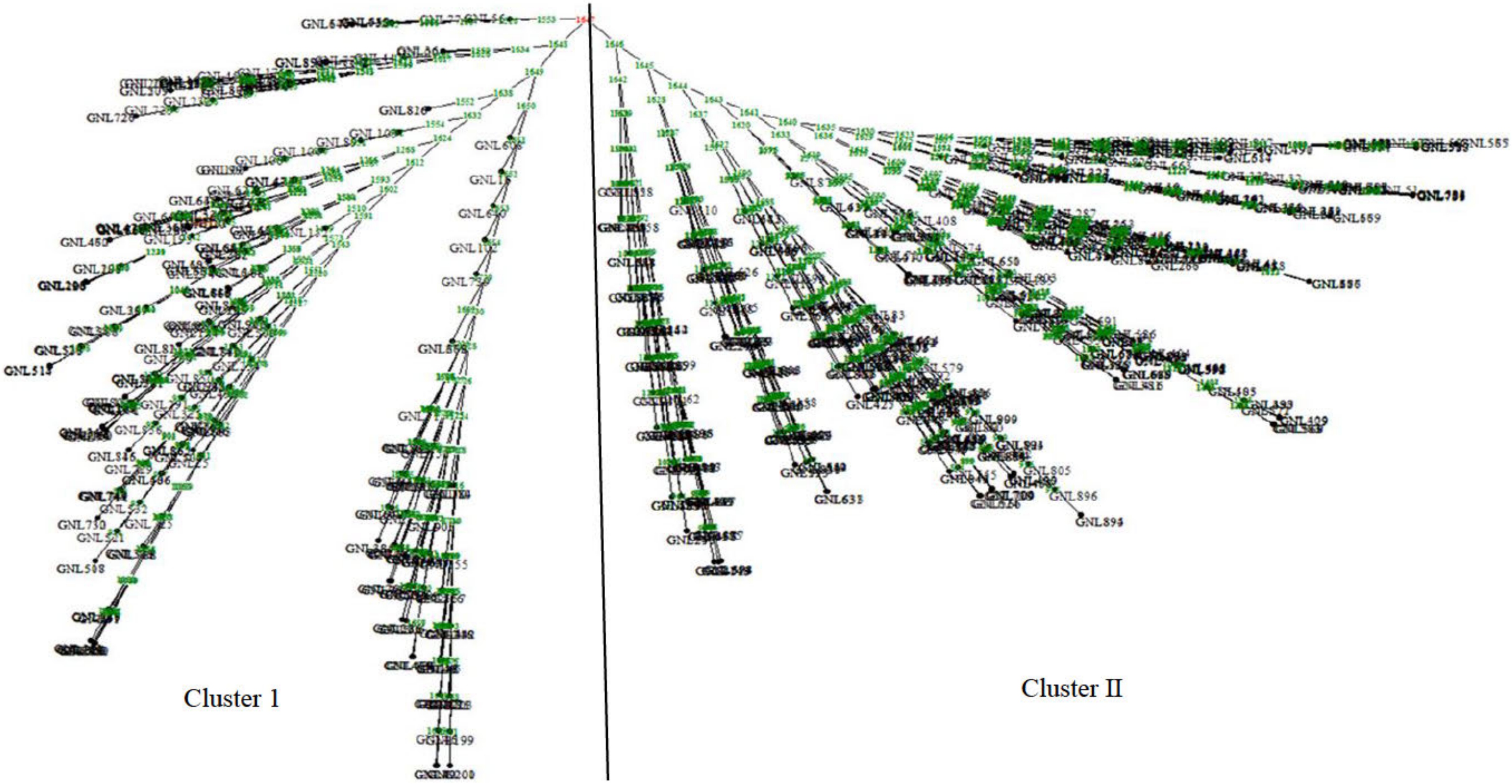

Figure 2.7 Phylogenetic relationships among 866 derived inbred lines, as inferred from 1484 SNP markers revealing distinct clusters and branches. See Table S 2.5 for codes of genotypes.

2.4 Discussion

Knowledge of genetic diversity, structure, and genetic relationships among elite inbred line populations of maize is vital for selecting genetically diverse and complementary lines for hybrid breeding and enhancing heterotic groups. Hybrid varieties are the best yielders, buffer biotic and abiotic stresses and achieve economic returns. Best cross combinations are selected based on maximum heterosis, which requires the development of homozygous elite lines and a subsequent assortment of contrasting heterotic patterns. Inbred lines with shared ancestry may lead to heterogeneous populations, limiting hybrid vigour. Inbred lines can be crossed, and a new generation of derived lines can be selected that are genetically complementary and distinct from other heterotic populations (Semagn et al., 2012; Adu et al., 2019).

High-throughput genotyping methods effectively determine and delineate the genetic relationships among inbred populations. Diagnostic molecular markers provide a high level of accuracy in identifying the genetic constitution of individuals to guide hybrid breeding (Lu et al., 2009; Zhou et al., 2009; Badu-Apraku et al., 2021). The SNP markers are widely used to assess genetic diversity and relationships (van Inghelandt et al., 2010; Singh et al., 2013), select breeding parents (Chen et al., 2011; Spindel et al., 2013), and identify novel genes linked to economically important traits (Rafalski, 2002; Mammadov et al., 2010). Also, SNPS are the genetic markers of choice due to several advantages, including their low cost per data point, widespread presence in the genome, specific location at genetic loci, co-dominance, and lower rates of genotyping errors (Mammadov et al., 2012; Semagn et al., 2012).

The present study evaluated the extent of genetic diversity, population structure, and genetic lineage of a maize germplasm panel consisting of 182 founder lines and 866 derived inbred lines using 1201 and 1484 SNP markers, respectively. The analysis revealed insights into allele frequency, which is crucial for understanding the extent of genetic variation among populations (Chacón et al., 2007). The major allele frequency varied from 0.50 to 0.99 (Table 2.3), revealing adequate genetic variation among the test populations for selection and potential for marker-assisted breeding programs. The mean MaF values for the 1201 and 1484 SNP markers were 0.82 and 0.85, respectively, indicating a high frequency of dominant alleles. The mean MAFs were 0.28 and 0.30 for the 1201 and 1484 markers, respectively (Table 2.3), indicating a moderate distribution of alleles among the inbred lines. This was consistent with previous reports by Semagn et al. (2012) and Suwarno et al. (2015), but lower than those reported by Muthusamy et al. (2014) and Oyekunle et al. (2015). Minor alleles indicate genetic variations of a specific gene or genetic marker crucial for preserving diversity within a population (Reif et al., 2004; Prassana, 2012; Govindaraj et al., 2015).

The assessed tropical elite maize lines had an average observed heterozygosity of 0.08 (Table 2.3), indicating a high degree of homozygosity and genetic stability. However, since inbred lines with heterozygosity > 5% are considered impure, some of the lines in this study may need additional selfing to reach the desired level of genetic purity. These findings align with Adu et al. (2019), who reported lower heterozygosity in early-maturing tropical maize inbred lines using SNP analysis. Mean heterozygosity values of 0.20 were reported by Yao et al. (2007) and Musundire et al. (2019). The polymorphism information content predicts the relevance of a genetic marker for linkage analysis (Meti et al., 2013; Zhang et al., 2016). In this study, the mean PIC values for the 1201 and 1484 markers were 0.37 and 0.41, respectively (Table 2.3), indicating that the SNP markers used were moderately informative. The moderate genetic polymorphism detected in this study is in line with the expected characteristics of bi-allelic SNP markers, which are limited to a maximum PIC value of 0.5. Nevertheless, the PIC values obtained in this study are still informative and can be used to evaluate the genetic diversity and relationships among the tropical elite maize lines (Eltaher et al., 2018; Luo et al., 2019), and the lower mutation rates of SNPs compared to other genetic markers (Coates et al., 2009). SNP markers are more precise in genetic analysis. However, SNPs show lower PIC values than other markers, such as SSRs (Heylar et al., 2011).

Genetic distance (GD) measures the genetic difference or dissimilarity among genotypes in a population and can be used to infer their genetic relationships (Oyekunle et al., 2015; Talabi et al., 2017; Adu et al., 2019). The present results showed considerable genetic variability among the inbred lines (Table 2.3). Genetic distances among the founder lines ranged from 0.006 to 0.44, with a mean of 0.25. The values suggest high genetic diversity and differentiation levels in the founder parental lines. The genetic distance values detected in the founder lines agree with previous studies in maize by Dao et al. (2014) and Adu et al. (2019). However, these values were lower than those reported by Lu et al. (2009) and van Inghelandt et al. (2010), who reported average genetic distance values of 0.32, as well as Yang et al. (2011) and Wu et al. (2015), who reported values of 0.37. The derived elite lines showed moderate genetic distances (mean of 0.13), indicating a reasonable degree of genetic variation among individuals while sharing significant genetic similarity (Table 2.3).

The SNP panels used in this study identified adequate genetic polymorphisms among and within the inbred line populations (Table 2.4). The low moderate diversity detected within the founder and derived inbred lines were attributable to genetic drift, founding effects, artificial selection, genetic recombination and linkage disequilibrium. A higher degree of genetic diversity at 97% that accounted for the within-population genetic variation was computed for the founder inbred line populations. In contrast, the variation among populations was low, at 3%, suggesting high gene flow due to outcrossing, genetic drift, founding effect and artificial selection. In the derived lines, among-population variation was 11.62%, while the within-population variation was higher at 88.38% ($p \leq 0.001$) signifying that the majority of genetic variation was partitioned within the population, suggesting a high level of genetic heterogeneity within the population. The high genetic diversity within the population exhibited by both elite sets of lines is a valuable asset for breeding and conservation purposes.

Population structure analysis determines the genetic ancestry of inbred lines (Nkatha et al., 2020; Dube et al., 2023). The genetic analysis using the SNP analysis delineated three subpopulations (K = 3) for the founder lines (Figure 2.3). The SNP markers effectively categorized the inbred lines into heterotic groups based on their source populations, grouping individuals with similar genetic backgrounds into the same subpopulations. The identified population groups guide breeding programs to select parental lines. The current results agree with Adu et al. (2019), who reported three subpopulations among 94 early maturing tropical maize inbred lines using SNP markers. Similarly, based on the neighbour-joining cluster analysis, the dendrogram (Figure 2.5) allocated the founder inbred lines into three genetic groups. Population analysis involving the 866 derived lines revealed two sub-populations (K = 2) (Figure 2.4). The inbred lines were assigned into heterotic groups based on similarity of ancestry and selection history. The genetic clustering based on the neighbour-joining cluster analysis supported the findings based on phylogenetic analysis. The grouping of the derived lines into two sub-populations is consistent with findings by Lu et al. (2009), who identified two groups (K=2) among 770 maize inbred lines using SNPs.

2.5 Conclusion

The present study assessed the genetic diversity and population structure comprising 182 founder lines and 866 derived inbred lines using diagnostic SNP markers and identified genetically unique lines for hybrid breeding. Higher genetic variations, at 97% and 88.38%, were attributed to within populations in the founder and derived lines, in that order. Population structure analysis identified three distinct genetic groups among founder lines and two among derived lines. Based on pairwise genetic comparison, the following founder and derived inbred lines were selected: G15NL337 and G15NL312 (Cluster 1), 15ARG152 and RGS-PL44 (Cluster 2), RGS-PL44 and 15ARG149 (Cluster 2), and RGS-PL33 and RGS-PL44 (Cluster 2), respectively. The selected lines are genetically distinct and recommended for marker-assisted hybrid maize breeding to exploit the frequency of beneficial alleles. The study identified novel genetically distant founder lines (i.e., 15ARG152 and RGS-PL44 and RGS-PL44 and 15ARG149) and derived lines (G15NL337 and G15NL312). The SNP markers identified with high polymorphism information content are valuable in genomic selection and genetic analysis and breeding. The core findings of the study are valuable references for maize breeding programs in Africa when using the current and related tropical-adapted populations.

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Table S 2.1 SNP markers used for genotyping 182 founder maize inbred lines.

CHR	SNP ID
1	ASSAY10208_00415, ASSAY1173_00197, ASSAY1290_00096, ASSAY1742_00143, ASSAY1751_00401, ASSAY1796_00123, ASSAY1796_00360, ASSAY1796_00457, ASSAY1822_00212, ASSAY1941_00169, ASSAY1994_00352, ASSAY2001_00126, ASSAY2001_00205, ASSAY2110_00115, ASSAY2165_00126, ASSAY2192_00127, ASSAY2214_00106, ASSAY2271_00573, ASSAY2483_00144, ASSAY2512_00251, ASSAY2654_00092, ASSAY2654_00154, ASSAY2654_00311, ASSAY2731_00185, ASSAY2757_00158, ASSAY2769_00153, ASSAY2803_00283, ASSAY2831_00093, ASSAY2831_00252, ASSAY2831_00371, ASSAY2911_00359, ASSAY2927_00166, ASSAY3117_00033, ASSAY3127_00216, ASSAY3135_00148, ASSAY3190_00322, ASSAY327_00151, ASSAY3292_00365, ASSAY3403_00064, ASSAY552_00279, ASSAY565_00217, ASSAY5847_00205, ASSAY6798_00109, ASSAY6817_00814, ASSAY731_00266, ASSAY748_00301, ASSAY777_00159, ASSAY8459_00493, ASSAY8459_01125, ASSAY8471_00031, ASSAY8517_00198, ASSAY8790_00455, ASSAY8795_00060, ASSAY8925_00294, ASSAY8926_00468, ASSAY925_00067, ASSAY931_00121, ASSAY948_00179, ASSAY955_00148, BGA00036A1_00150, BGA00036A1_00580, BGA00085F4R7_00215, BGA00131F2R2A1_00351, BGA00140F1R1_00647, EPG00057F3R3_00140, EPG00057F3R3_00337, EPG00057F3R3_00614, EPG00057F3R3_00800, KWS00021F3R3_00417, LIM00048_00345, LIM00049F5R5_00364, LIM00132_00027, LIM00133_00152, PUT-163A-31997542-2011, PUT-163A-60344363-2515, PUT-163A-60350073-2631, PUT-163A-71305340-3082, PUT-163A-76012177-3730, PUT-163A-86470789-4503, PUT-163A-88747038-4526, PZA00468.8, PZA02359.10, PZA02957.5, PZA03557.1, PZB01915.1, PZE-101009163, PZE-101023852, PZE-101024808, PZE-101033622, PZE-101035737, PZE-101039207, PZE-101048857, PZE-101049091, PZE-101057118, PZE-101068816, PZE-101160171, PZE-101160748, PZE-101162399, PZE-101163301, PZE-101163849, PZE-101163883, PZE-10116815, PZE-101197298, PZE-101198605, PZE-101198781, PZE-101199832, PZE-101200344, PZE-101201219, PZE-101201492, PZE-101203040, PZE-101203924, PZE-101205031, PZE-101205714, PZE-101206027, PZE-101206252, PZE-101207067, PZE-101207166, PZE-101207892, PZE-101208157, PZE-101208921, PZE-101209157, PZE-101210110, PZE-101210734, PZE-101211692, PZE-10121411, PZE-101214804, PZE-101215143, PZE-101215699, PZE-101216104, PZE-101217024, PZE-101218183, PZE-101221171, PZE-101222284, PZE-101224367, PZE-101225113, PZE-101225132, PZE-101226725, PZE-101227145, PZE-101228665, PZE-101229195, PZE-101229632, PZE-101230194, PZE-101230537, PZE-101232138, PZE-101233856, PZE-101234638, PZE-101236600, PZE-101236759, PZE-101237912, PZE-101238691, PZE-101247063, PZE-101249551, PZE-101249703, PZE-101251539, PZE-101252431, PZE-101256110, PZE-101256470, RAG00037F1R1_00354, RAG00037F1R1_00408, RAG00037F1R1_00562, RAG00119F4R5_00426, RAG00119F4R5_00436, RAG00119F4R5_00507, RAG00148F1R2_00334, RAG00194F2R2_00540, SYN14016, SYN15630, SYN16862, SYN16865, SYN2024, SYN21366, SYN21642, SYN27356, SYN27374, SYN297, SYN38906, SYN38939, SYN8233, SYN8236, ZM000417_0480, ZM000434_1802, ZM001195_0628, ZM001629_0989, ZM001719_0119, ZM002382_0505, ZM002383_0403, ZM002689_0540, ZM002776_0860, ZM002985_0179, ZM003044_1290, ZM003900_0697, ZM004071_0670, ZM004694_0326, ZM005800_0217, ZM006103_0363, ZM006103_0446, ZM006288_1134, ZM006877_0585, ZM007810_0794, ZM008178_0127, ZM008698_0833, ZM008740_0593, ZM009424_0548, ZM009680_0375, ZM010129_0558, ZM010322_0169, ZM010458_0571, ZM010563_0342, ZM010766_1155, ZM011281_0570, ZM011857_0402, ZM012324-0461
2	ASSAY10122_00805 ASSAY1044_00085 ASSAY1114_00580 ASSAY1114_00781 ASSAY1119_00037 ASSAY1247_00558 ASSAY1247_00728 ASSAY1733_00295 ASSAY1758_00513 ASSAY1914_00276 ASSAY1938_00167 ASSAY1938_00230 ASSAY2043_00246 ASSAY2071_00243 ASSAY2073_00097 ASSAY2073_00173 ASSAY2147_00289 ASSAY2155_00070 ASSAY2190_00350 ASSAY2213_00210 ASSAY2265_00173 ASSAY2342_00148 ASSAY2393_00257 ASSAY2570_00292 ASSAY2575_00306 ASSAY2631_00244 ASSAY2794_00355 ASSAY2977_00175 ASSAY2977_00251 ASSAY3002_00282 ASSAY3011_00156 ASSAY3189_00146 ASSAY3204_00230 ASSAY3321_00088 ASSAY3372_00119 ASSAY464_00037 ASSAY533_00323 ASSAY560_00580 ASSAY591_00467 ASSAY5927_00412 ASSAY5987_00294 ASSAY6016_00056 ASSAY715_00153 ASSAY745_00164 ASSAY8505_00418 ASSAY8505_00866 ASSAY8699_00172 ASSAY8699_00692 ASSAY872_00169 ASSAY8869_00366 ASSAY8908_00230 ASSAY8922_00092 ASSAY8924_00420 BGA00027F5R5_00436 BGA00118F4R4_00053 BGA00212F3R3_00238 BGA00234F2R2_00073 BGA00306F1R1_00480 BGA00370F1R1_00095 EPG00113_00630 EPG00139F2R2_00446 EPG00210F2R2_00262 LIM00097_00903 LIM00151F199R199_00376 PZA02727.1 PZE-102063478 PZE-102067230 PZE-102069040 PZE-102069195 PZE-102070420 PZE-102084771 PZE-102098807 PZE-102183145 PZE-102184919 PZE-102185011 PZE-102185564 PZE-102185952 PZE-102191279 PZE-102192375 PZE-102192647 PZE-102193034 PZE-102194180 PZE-110090076 RAG00169F2R2_00214 RAG00210F2R2_00088 SYN14631 SYN18301 SYN20670 SYN28307 SYN34400 ZM000641_0697 ZM001125_1218 ZM001293_0732 ZM001525_0541 ZM001679_0624 ZM002806_0674 ZM003338_0691 ZM003429_0308 ZM004340_0418 ZM005597_1446 ZM005925_0931 ZM006229_0205 ZM006881_0736 ZM006881_1075 ZM007140_0341 ZM007489_0332 ZM007489_0517 ZM007621_0429 ZM007621_0513 ZM008336_0716 ZM009029_0412 ZM009592_0683ZM010663_0466

Table S 2.1 continued

CHR	SNP ID
3	ASSAY1041_00081 ASSAY1155_00094 ASSAY1491_00173 ASSAY1655_00046 ASSAY1709_00246 ASSAY1727_00379 ASSAY1790_00152 ASSAY1790_00238 ASSAY1792_00090 ASSAY1850_00038 ASSAY1862_00241 ASSAY1968_00249 ASSAY2040_00240 ASSAY2209_00431 ASSAY2360_00260 ASSAY2375_00184 ASSAY2375_00199 ASSAY2449_00266 ASSAY2467_00365 ASSAY2480_00293 ASSAY2520_00222 ASSAY2602_00191 ASSAY2762_00058 ASSAY2895_00094 ASSAY2939_00073 ASSAY2947_00197 ASSAY2994_00089 ASSAY3056_00259 ASSAY3151_00129 ASSAY3308_00312 ASSAY3314_00146 ASSAY3382_00159 ASSAY345_02832 ASSAY5844_00439 ASSAY585_00088 ASSAY5861_00269 ASSAY606_00412 ASSAY6474_00916 ASSAY6802_00267 ASSAY817_00190 ASSAY8485_00254 ASSAY8509_00130 ASSAY8509_00643 ASSAY8524_00236 ASSAY8526_00249 ASSAY8704_00418 ASSAY8786_00045 ASSAY8786_00094 ASSAY8786_00290 ASSAY8791_00526 ASSAY8791_00691 ASSAY8791_00725 ASSAY926_00114 ASSAY942_00058 BGA00218F6R6_00295 BGA00369F1R1_00442 BGA00448F1R1_00615 EPG00011F2R2_00189 EPG00011F2R2_00375 EPG00158F1R1_00266 LIM00091_00390 PUT-163A-78113073-4314 PZB00228.3 PZE-103025094 PZE-103034532 PZE-103045772 PZE-103072561 PZE-103076837 PZE-103077185 PZE-103077912 PZE-103078702 PZE-103083512 PZE-103083722 PZE-103084005 PZE-103085573 PZE-103089292 PZE-103090476 PZE-103091693 PZE-103100115 PZE-103102170 QTL3MADSF21R21_00167 QTL3MADSF21R21_00211 RAG00056F1R1_00050 RAG00066F1R1_00497 RAG00089F2R2_00133 RAG00102F1R1_00242 RAG00136F2R2_00398 ZM000452_0355 ZM000515_1189 ZM000583_1178 ZM001101_1649 ZM001140_0258 ZM001683_0381 ZM001754_0273 ZM001839_1234 ZM002328_0630 ZM002911_1254 ZM003785_0433 ZM004333_1181 ZM004456_0637 ZM006120_1056 ZM006442_0209 ZM007184_0534 ZM007594_0420 ZM008693_0358 ZM008759_0264 ZM008789_0123 ZM008829_0535 ZM009167_0320 ZM009707_0213 ZM009949_0423 ZM011057_0368 ZM011243_0160 ZM011785_0468
4	ASSAY1185_00104 ASSAY1239_00197 ASSAY1478_00151 ASSAY1575_00216 ASSAY1671_00521 ASSAY1752_00165 ASSAY1766_00265 ASSAY1836_00272 ASSAY1884_00203 ASSAY1947_00152 ASSAY1983_00589 ASSAY2205_00233 ASSAY2266_00235 ASSAY2292_00417 ASSAY2405_00296 ASSAY2405_00395 ASSAY2490_00071 ASSAY2566_00059 ASSAY2628_00297 ASSAY2682_00132 ASSAY2787_00275 ASSAY2791_00512 ASSAY294_00734 ASSAY294_01086 ASSAY2998_00221 ASSAY3119_00494 ASSAY3121_00229 ASSAY3142_00055 ASSAY3149_00105 ASSAY3149_00138 ASSAY3177_00359 ASSAY3407_00142 ASSAY5872_00203 ASSAY5982_00454 ASSAY645_00091 ASSAY8521_00453 ASSAY8529_00502 ASSAY8860_00096 BGA00099F2R2_00340 BGA00099F2R2_00475 BGA00425F1R1_00042 BGA00425F1R1_00394 EPG00128F3R3_00639 KWS00017F1R1_00083 KWS00020F7R7_00170 LIM00021A1_00677 LIM00032F2R2_00435 LIM00057F3R5_00436 LIM00070F1R1_00352 PZE-104073794 PZE-104076811 PZE-104078833 PZE-104079748 PZE-104081085 PZE-104082522 PZE-104083719 PZE-104086265 PZE-104087964 PZE-104089424 PZE-104090186 PZE-104093384 PZE-104094429 PZE-104096416 PZE-104099220 PZE-104113793 PZE-104117537 PZE-104157695 RAG00053A1_03158 RAG00170F1R1_00414 SYN1891 SYN36306 ZM003235_0672 ZM003802_0593 ZM004804_0307 ZM004938_1249 ZM005435_0536 ZM007188_0227 ZM007280_0528 ZM007486_0865 ZM009060_1185 ZM011028_0260ZM011278_0547 ZM012058_0561 ZM012531-0288
5	ASSAY10179_00299 ASSAY1034_00221 ASSAY1079_00139 ASSAY1278_00173 ASSAY1283_00016 ASSAY1283_00276 ASSAY1323_00041 ASSAY1460_00385 ASSAY1481_00174 ASSAY1507_00119 ASSAY1679_00065 ASSAY1679_00233 ASSAY1825_00090 ASSAY1903_00108 ASSAY1943_00172 ASSAY2194_00155 ASSAY2224_00379 ASSAY2236_00266 ASSAY2442_00291 ASSAY2518_00368 ASSAY2623_00253 ASSAY2690_00290 ASSAY2783_00362 ASSAY2846_00159 ASSAY3165_00391 ASSAY3281_00448 ASSAY3281_00537 ASSAY3316_00259 ASSAY3370_00098 ASSAY3391_00366 ASSAY3391_00429 ASSAY514_00169 ASSAY543_00190 ASSAY543_00195 ASSAY545_00248 ASSAY550_00069 ASSAY5925_00055 ASSAY6006_00305 ASSAY677_00139 ASSAY705_00179 ASSAY759_00095 ASSAY851_00163 ASSAY8528_00307 ASSAY8733_00321 ASSAY8733_00475 ASSAY882_00189 ASSAY8976_00390 AVE00038_00142 AVE00055_00213 BGA00008F5R5_00382 BGA00022F7R7_00391 BGA00041A1_00051 BGA00041A1_00716 BGA00041A1_01728 BGA00132F1R1A1_00441 BGA00137A1_00132 BGA00180F1R1_00213 BGA00201F1R1_00428 BGA00469F1R1_00634 EPG00118_00267 EPG00118_00398 LIM00003A1_00132 LIM00003A1_00512 LIM00003A2_00059 LIM00118F236R236_00041 PUT-163A-60350211-2639 PUT-163A-76291488-3952 PUT-163A-78086550-4187 PZA00255.17 PZB00182.1 PZE-105017042 PZE-105020816 PZE-105021941 PZE-105022201 PZE-105022328 PZE-105022450 PZE-105022509 PZE-105022516 PZE-105022751 PZE-105024460 PZE-105024579 PZE-105024985 PZE-105025087 PZE-105025224 PZE-105026164 PZE-105026330 PZE-105027872 PZE-105029444 PZE-105030407 PZE-105031034 PZE-105031086 PZE-105031696 PZE-105031717 PZE-105033399 PZE-105033935 PZE-105042856 PZE-105042963 PZE-105046237 PZE-105046457 PZE-105064517 PZE-105073571 PZE-105073579 PZE-105074639 PZE-105076159 PZE-105078900 PZE-105078909 PZE-105081557 PZE-105082311 PZE-105082431 PZE-105082736 PZE-105083429 PZE-105086726 PZE-105089936 PZE-105090165 PZE-105091062 PZE-105091854 PZE-105092539 PZE-105093999 PZE-105096172 PZE-105097260 PZE-105101487 PZE-105102442 PZE-105103245 PZE-105105756 PZE-105105940 PZE-105106949 PZE-105107897 PZE-105109345 PZE-105111088 PZE-105115780 PZE-105117617 PZE-105118656 PZE-105122814 PZE-105123635 PZE-105126583 RAG00081F3R3_00237 RAG00198F3R4_00526 SYN23466 SYN27136 SYN903 SYN9877 ZM000340_0671 ZM000390_0106 ZM000390_0332 ZM000480_0999 ZM001117_2665 ZM001260_1203 ZM001312_0511 ZM002063_0840 ZM002399_1206 ZM003472_2272 ZM004846_0778 ZM005379_0825 ZM005594_1051 ZM006776_0441 ZM006987_0455 ZM007333_0355 ZM007334_0207 ZM008222_0813 ZM008357_0643 ZM008589_0259 ZM009245_0373 ZM009878_0471 ZM010486_0724 ZM011982_1108
6	ASSAY1121_00089 ASSAY1176_00186 ASSAY1414_00933 ASSAY1431_00504 ASSAY1557_00064 ASSAY1569_00169 ASSAY1747_00024 ASSAY1780_00174 ASSAY1821_00182 ASSAY2019_00403 ASSAY2149_00321 ASSAY2288_00756 ASSAY2460_00193 ASSAY2688_00172 ASSAY2887_00179 ASSAY3036_00225 ASSAY3246_00100 ASSAY3246_00169 ASSAY3328_00559 ASSAY3354_00231 ASSAY3356_00059 ASSAY4698_00387 ASSAY470_00254 ASSAY551_00218 ASSAY6507_00641 ASSAY651_00398 ASSAY912_00159 ASSAY984_00356 BGA00114A1_00591 BGA00122F1R1_00051 EPG00065F3R3_00496 EPG00220F1R1_00270 KWS00031F1R1_00359 LIM00134A1_00705 LIM00147F255R255_00200 PUT-163A-149095867-900 PUT-163A-60339740-2390 PUT-163A-88754607-4573 PZE-106000516 PZE-106000855 PZE-106003964 PZE-106004214 PZE-106004598 PZE-106005094 PZE-106007130 PZE-106007950 PZE-106008070 PZE-106008513 PZE-106008760 PZE-106011274 PZE-106013072 PZE-106017319 PZE-106017695 PZE-106017960 PZE-106019566 PZE-106020586 PZE-106022944 PZE-106023074 PZE-106025255 PZE-106026913 PZE-106027037 PZE-106031939 PZE-106035034 PZE-106036132 PZE-106037023 PZE-106037935 PZE-106039805 PZE-106041765 PZE-106042213 PZE-106045060 PZE-106053038 PZE-106053973 PZE-106056051 PZE-106056472 PZE-

Table S 2.1 continued

CHR	SNP ID
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7	ASSAY10216_00198 ASSAY10216_00345 ASSAY1223_00125 ASSAY1315_00053 ASSAY1456_00072 ASSAY1463_00069 ASSAY1519_00068 ASSAY1530_00061 ASSAY1530_00475 ASSAY1612_00117 ASSAY1768_00296 ASSAY1784_00267 ASSAY2158_00076 ASSAY2477_00443 ASSAY3062_00266 ASSAY3231_00263 ASSAY3283_00258 ASSAY3329_00186 ASSAY3331_00086 ASSAY4658_00368 ASSAY532_00155 ASSAY5895_00303 ASSAY5934_00433 ASSAY5968_00269 ASSAY5981_00227 ASSAY640_00070 ASSAY706_00162 ASSAY737_00082 ASSAY753_00099 ASSAY822_00141 ASSAY8523_00512 ASSAY8734_00100 ASSAY8734_01030 ASSAY8792_00171 ASSAY8857_00501 ASSAY8857_00917 ASSAY8896_00155 ASSAY8899_00146 ASSAY8918_00231 ASSAY986_00302 BGA00181A1_00649 BGA00213F2R2_00291 BGA00244F7R7_00145 EPG00154F2R2_00046 PZE-107085004 RAG00084F2R2_00552 RAG00135F1R1_00376 SYN23679 ZM000443_0535 ZM000468_0476 ZM000654_0808 ZM000654_0964 ZM000705_0554 ZM000843_1652 ZM001181_1292 ZM001181_1348 ZM002694_0755 ZM003358_0956 ZM003582_0482 ZM006260_0546 ZM006627_1161 ZM006954_0421 ZM006954_0886 ZM007017_0435 ZM007057_1730 ZM007565_0684 ZM007718_0376 ZM011672_0804 ZM011672_0894 ZM011877_0594
8	ASSAY10160_00400 ASSAY10162_00160 ASSAY1024_00150 ASSAY1065_00162 ASSAY1065_00775 ASSAY1341_00252 ASSAY1399_00036 ASSAY1526_00395 ASSAY1588_00036 ASSAY1625_00118 ASSAY1660_00126 ASSAY1851_00187 ASSAY1928_00371 ASSAY1933_00182 ASSAY1977_00072 ASSAY2101_00193 ASSAY2183_00088 ASSAY2636_00222 ASSAY2814_00145 ASSAY2843_00233 ASSAY2921_00112 ASSAY3001_00298 ASSAY3010_00157 ASSAY475_00074 ASSAY498_00255 ASSAY502_00297 ASSAY558_00116 ASSAY5884_00022 ASSAY592_00056 ASSAY617_00489 ASSAY617_00569 ASSAY631_00241 ASSAY6521_00033 ASSAY6521_00611 ASSAY6799_00056 ASSAY788_00294 ASSAY869_00227 ASSAY8784_00297 ASSAY8851_00052 ASSAY8872_00327 ASSAY8941_00279 ASSAY8943_00094 ASSAY8943_00378 ASSAY8946_00112 ASSAY965_00233 AVE00086_00083 BGA00002A2_01006 BGA00005F5R5_00197 BGA00107F3R3_00229 BGA00121A1_00023 BGA00134A1_00059 BGA00275A1_00226 BGA00277A1_00151 BGA00277A1_00161 EPG00126F1R1_00730 EPG00143F1R1_00721 EPG00147F2R2_00064 EPG00147F2R2_00086 EPG00147F2R2_00260 EPG00213F3R3_00510 EPG00214F3R3_00051 EPG00214F3R3_00348 LIM00092_00296 LIM00092_00532 LIM00092_00663 LIM00092_00695 PHM15278.6 PUT-163A-29576864-1790 PUT-163A-78076151-4108 PZB01977.1 PZE-108000104 PZE-108000665 PZE-108001260 PZE-108001699 PZE-108002130 PZE-108002202 PZE-108010180 PZE-108015266 PZE-108018453 PZE-108018598 PZE-108018898 PZE-108019359 PZE-108019468 PZE-108019541 PZE-108019544 PZE-108019557 PZE-108019790 PZE-108019862 PZE-108019866 PZE-108020413 PZE-108020433 PZE-108020588 PZE-108020972 PZE-108022079 PZE-108022229 PZE-108022547 PZE-108022574 PZE-108022596 PZE-108024170 PZE-108025072 PZE-108025073 PZE-108026312 PZE-108026419 PZE-108026433 PZE-108029721 PZE-108032449 PZE-108033822 PZE-108036270 PZE-108036821 PZE-108039809 PZE-108057745 PZE-108066888 PZE-108067005 PZE-108070106 PZE-108070885 PZE-108072647 PZE-108072804 PZE-108073083 PZE-108073510 PZE-108074975 PZE-108076380 PZE-108078659 PZE-108078820 PZE-108080842 PZE-108081656 PZE-108084145 PZE-108084629 PZE-108086288 PZE-108086766 PZE-108087618 PZE-108090243 PZE-108090463 PZE-108090521 PZE-108091190 PZE-108091439 PZE-108092412 PZE-108092596 PZE-108093787 PZE-108094590 PZE-108094808 PZE-108096469 PZE-108096732 PZE-108096791 PZE-108098094 PZE-108098580 PZE-108098682 PZE-108099425 PZE-108100090 PZE-108100174 PZE-108100418 PZE-108100984 PZE-108101323 PZE-108101551 PZE-108101966 PZE-108102250 PZE-108102490 PZE-108102698 PZE-108103023 PZE-108103185 PZE-108103365 PZE-108103951 PZE-108104106 PZE-108104357 PZE-108104589 PZE-108105561 PZE-108106506 PZE-108106737 PZE-108107072 PZE-108107190 PZE-108107270 PZE-108107671 PZE-108108491 PZE-108108690 PZE-108108866 PZE-108110041 PZE-108112715 PZE-108115144 RAG00011F5R5_00359 RAG00058F1R1_00289 SYN10052 SYN10324 SYN11271 SYN11424 SYN15862 SYN18639 SYN18660 SYN19605 SYN25100 SYN31344 SYN32654 SYN35861 SYN9035 SYN9919 SYNGENTA7016 ZM000296_0502 ZM000316_0350 ZM001079_0678 ZM004075_0404 ZM004820-0602 ZM005020_0445 ZM005524_0467 ZM005524_0656 ZM005912_0452 ZM006265_1111 ZM007357_0390 ZM007733_0710 ZM008345_0433 ZM008615_0640 ZM009376_0207 ZM009801_0336 ZM010237_1012 ZM011404_0762 ZM011420_0378 ZM011878_0364 ZM012374-0236
9	ASSAY1094_00220 ASSAY1252_00250 ASSAY1338_00040 ASSAY1397_00291 ASSAY1839_00449 ASSAY2048_00477 ASSAY2145_00113 ASSAY2339_00340 ASSAY2339_00650 ASSAY2359_00228 ASSAY2474_00068 ASSAY271_00401 ASSAY289_00638 ASSAY2961_00145 ASSAY2984_00048 ASSAY344_04453 ASSAY452_00222 ASSAY4692_00233 ASSAY4692_00320 ASSAY569_00127 ASSAY864_01047 ASSAY885_00222 ASSAY8868_00369 ASSAY980_00346 BGA00117F2R2_00083 BGA00147F2R2_00285 BGA00293F1R1_00264 EPG00138F2R2_00148 LIM00066F4R4_00028 LIM00090_00579 LIM00095_00651 LIM00143F227R227_00313 PZE-109003658 PZE-109003659 PZE-109003902 PZE-109004430 PZE-109004775 PZE-109008703 PZE-109008839 PZE-109009222 PZE-109098623 PZE-109121548 RAG00024F1R1_00146 RAG00206A1_00677 SYN19748 ZM000789_0313 ZM002285_0781 ZM003097_0921 ZM003608_0684 ZM005112_1024 ZM005314_0525 ZM005887_0752 ZM007213_0812 ZM007589_1362 ZM008236_0731 ZM009639_0328 ZM010395_0927 ZM010491_0457 ZM010851_0384
10	ASSAY1000_00086 ASSAY1021_00410 ASSAY1957_00055 ASSAY1957_00373 ASSAY2305_00174 ASSAY2523_00222 ASSAY2523_00306 ASSAY2758_00222 ASSAY2828_00137 ASSAY2828_00185 ASSAY2861_00356 ASSAY2993_00122 ASSAY3038_00072 ASSAY3073_00150 ASSAY3169_00133 ASSAY3194_00076 ASSAY6484_00068 ASSAY6768_00407 ASSAY6768_00733 ASSAY710_00348 ASSAY718_00136 ASSAY8806_00651 ASSAY8890_00338 ASSAY950_00069 BGA00128F3R3_00291 BGA00130F1R1_00507 BGA00278F1R1_00520 BGA00278F1R1_00530 EPG00009A1_00481 EPG00009A1_00582 EPG00030F3R3_00036 EPG00119_00588 EPG00119F3R3_00021 PUT-163A-71444135-3357 PZA03570.1 PZE-110082173 PZE-110082278 PZE-110085688 PZE-110085763 PZE-110086326 PZE-110086343 PZE-110090887 PZE-110092938 PZE-110093304 PZE-110097238 PZE-110103156 PZE-110106915 SYN17783 SYN18725 SYN21905 SYN36714 SYN8162 ZM000849_0204 ZM001954_0751 ZM002884_0156 ZM005754_0332 ZM005950_0320 ZM007018_0531 ZM007148_0518 ZM007217_0521 ZM009509_0438 ZM011808_0431

CHR=chromosome number, SNP ID=single nucleotide polymorphic marker identity

Table S 2.2 SNPs used for genotyping 866 derived tropical maize inbred lines.

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Table S 2.2 continued

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CHR=chromosome number, SNP ID=single nucleotide polymorphic marker identity

Table S 2.3 Codes for the 182-founder parental inbred lines of maize.

Line Code	Code description
1 to 10	RGS-PL65, RGS-PL08, RGS-PL09, RGS-PL68, RGS-PL63, RGS-PL62, RGS-PL38, RGS-PL53, 16ARG16812, RGS-PL20
11 to 20	RGS-PL10, RGS-PL58, RGS-PL06, 15ARG119, 15ARG142, 15ARG143, 15ARG148, 15ARG149, 15ARG151, 15ARG175
21 to 30	RGS-PL28,16ARG16786, 16ARG16788, 16ARG16791, 16ARG16792, 16ARG16804,16ARG16816, RGS-PL23, RGS-PL48, RGS-PL52
31 to 40	15ARG104, 15ARG176, RGS-PL30, 16ARG16798, 16ARG16815, RGS-PL54, RGS-PL19, RGS-PL17, 15ARG112, 15ARG127
41 to 50	15ARG129, 15ARG152, RGS-PL60, RGS-PL13, 16ARG16782, 16ARG16814, RGS-PL07, RGS-PL25, 15ARG111, 15ARG114
51 to 60	15ARG132, 15ARG140, 15ARG161, RGS-PL66, RGS-PL11, RGS-PL71, RGS-PL36, RGS-PL01, 16ARG16784, 16ARG16795
61 to 70	RGS-PL44, 15ARG110, 15ARG123, 15ARG164, RGS-PL31, RGS-PL29, RGS-PL43, 16ARG16794, RGS-PL47, RGS-PL69
71 to 80	15ARG117, 15ARG128, 15ARG158, 15ARG160, 15ARG173, 16ARG180, 16ARG16790, 16ARG16802, 16ARG16806, 16ARG16811
81 to 90	RGS-PL50, RGS-PL03, RGS-PL59, 15ARG131, 15ARG137, 15ARG147, 15ARG154, 15ARG155, 15ARG159, 15ARG165
91 to 100	15ARG167, 15ARG174, RGS-PL27, RGS-PL33, 16ARG178, 16ARG16803, 16ARG16807, RGS-PL18, RGS-PL24, 15ARG106
101 to 110	15ARG107, 15ARG116, 15ARG125, 15ARG126, 15ARG130, 15ARG144, 15ARG153, 15ARG171, RGS-PL05, RGS-PL45
111 to 120	16ARG16781, 15ARG121, 15ARG124, 15ARG157, 15ARG168, RGS-PL64, 16ARG16789, 16ARG16796, 16ARG16797, 16ARG16801
121 to 130	16ARG16805, RGS-PL56, RGS-PL55, RGS-PL15, RGS-PL21, RGS-PL02, 15ARG145, 15ARG163, 15ARG172, 16ARG181
131 to 140	16ARG16800, 15ARG109, 15ARG120, 15ARG122, 15ARG136, 15ARG138, 15ARG166, 15ARG177, RGS-PL14, 16ARG16785
141 to 150	16ARG16809, 16ARG16817, RGS-PL26, RGS-PL16, RGS-PL40 15ARG113, 15ARG115, 15ARG150, 15ARG156, RGS-PL57
151 to 160	RGS-PL61, RGS-PL49, RGS-PL37, 16ARG16793, RGS-PL12, RGS-PL22, RGS-PL04, RGS-PL39, 15ARG105, 15ARG133
161 to 170	15ARG135, 15ARG139, 15ARG169, RGS-PL70, RGS-PL42, 16ARG16799, 16ARG16808, 15ARG103, 15ARG146, 15ARG170
171 to 182	RGS-PL67, 16ARG179, 16ARG16787, 16ARG16810, 16ARG16813, RGS-PL41, RGS-PL46, 15ARG108, 15ARG118, 15ARG134, 15ARG141, 15ARG162

Table S 2.4 Distribution of the 182 founder parental lines of maize into three subpopulations.

Cluster	Line
1	15ARG164 RGS-PL19 RGS-PL12 15ARG120 15ARG170 15ARG139 15ARG151 15ARG105 15ARG103 15ARG135 15ARG111 15ARG138 15ARG104 RGS-PL50 15ARG131 15ARG159 RGS-PL39 15ARG122 15ARG142 15ARG140 15ARG158 15ARG173 15ARG145 RGS-PL01 15ARG167 15ARG161 RGS-PL47 15ARG163 15ARG143 15ARG148 15ARG147 15ARG112 15ARG127 15ARG129 15ARG121 15ARG110 15ARG123 15ARG117
2	RGS-PL65 RGS-PL08 RGS-PL09 RGS-PL68 RGS-PL63 RGS-PL62 RGS-PL38 RGS-PL53 RGS-PL20 RGS-PL10 RGS-PL58 RGS-PL06 RGS-PL23 RGS-PL48 RGS-PL52 15ARG176 RGS-PL30 RGS-PL54 RGS-PL17 RGS-PL60 RGS-PL13 RGS-PL07 RGS-PL25 15ARG132 RGS-PL66 RGS-PL11 RGS-PL71 RGS-PL36 RGS-PL44 RGS-PL31 RGS-PL29 RGS-PL43 RGS-PL69 15ARG128 15ARG160 RGS-PL03 15ARG137 15ARG149 15ARG154 15ARG155 15ARG165 15ARG174 RGS-PL27 RGS-PL33 16ARG178 RGS-PL18 RGS-PL24 15ARG106 15ARG107 15ARG116 15ARG125 15ARG126 15ARG130 15ARG144 15ARG153 15ARG171 RGS-PL05 RGS-PL45 15ARG152 15ARG124 15ARG157 15ARG168 RGS-PL64 RGS-PL56 RGS-PL55 RGS-PL15 RGS-PL21 RGS-PL02 15ARG172 15ARG109 15ARG136 15ARG166 15ARG177 RGS-PL14 RGS-PL26 RGS-PL16 RGS-PL40 15ARG113 15ARG115 15ARG150 15ARG156 RGS-PL57 RGS-PL61 RGS-PL49 RGS-PL37 RGS-PL22 RGS-PL04 15ARG133 15ARG169 RGS-PL70 RGS-PL42 15ARG146 RGS-PL67 RGS-PL41 RGS-PL46 15ARG108 15ARG118 15ARG134 15ARG141 15ARG162
3	RGS-PL28 16ARG16787 15ARG114 16ARG16817 15ARG119 16ARG16792 15ARG175 16ARG16815 16ARG16814 16ARG16811 RGS-PL59 16ARG16804 16ARG16782 16ARG16786 16ARG16798 16ARG16784 16ARG16795 16ARG16794 16ARG16781 16ARG16796 16ARG16797 16ARG16801 16ARG16803 16ARG16807 16ARG16785 16ARG16809 16ARG16812 16ARG16791 16ARG16800 16ARG16793 16ARG16799 16ARG16808 16ARG16810 16ARG16813 16ARG16806 16ARG16805 16ARG179 16ARG16789 16ARG16790 16ARG16788 16ARG16816 16ARG16802 16ARG181 16ARG180

Table S 2.5 Two clusters of the 866 derived maize inbred lines based on SNP analysis.

Cluster	Genotypes
1	G15NL357 G15NL288 G16NL42 G16NL71 G16NL83 G16NL93 G16NL94 G16NL95 G16NL130 G16NL153 G17NL182 G17NL184 G17NL225 G18NL262 G18NL264 G15NL348 G15NL350 G15NL351 G15NL360 G15NL299 G18NL206 G16NL89 G16NL91 G16NL97 G16NL105 G16NL106 G16NL114 G16NL132 G16NL156 G17NL166 G17NL174 G17NL175 G17NL191 G18NL276 G15NL04 G15NL32 G15NL36 G15NL346 G15NL291 G15NL295 G16NL72 G16NL99 G16NL109 G16NL119 G16NL126 G17NL157 G17NL196 G17NL213 G17NL218 G17NL222 G17NL228 G17NL237 G18NL246 G15NL27 G15NL362 G15NL313 G15NL361 G15NL283 G16NL48 G16NL59 G16NL81 G16NL125 G16NL150 G17NL159 G17NL192 G17NL194 G17NL207 G17NL214 G17NL505 G17NL234 G18NL274 G15NL16 G15NL308 G15NL281 G15NL297 G15NL298 G16NL38 G16NL46 G16NL60 G16NL61 G16NL684 G16NL73 G16NL100 G16NL123 G16NL124 G16NL149 G17NL202 G17NL221 G17NL231 G17NL233 G18NL253 G18NL263 G15NL367 G15NL368 G15NL310 G15NL330 G15NL331 G15NL344 G15NL354 G15NL294 G16NL62 G16NL65 G16NL67 G16NL78 G16NL85 G16NL86 G15NL12 G15NL14 G15NL31 G15NL306 G15NL312 G15NL320 G16NL43 G16NL54 G16NL57 G16NL63 G16NL107 G17NL163 G17NL170 G17NL178 G17NL179 G17NL200 G17NL193 G17NL209 G17NL210 G17NL212 G17NL220 G16NL136 G16NL142 G18NL266 G15NL05 G15NL26 G15NL364 G15NL370 G15NL309 G15NL334 G15NL336 G15NL342 G16NL50 G16NL66 G16NL75 G16NL96 G16NL98 G16NL133 G17NL187 G17NL189 G17NL198 G17NL199 G17NL215 G18NL248 G18NL249 G18NL257 G18NL261 G18NL270 G18NL280 G15NL03 G15NL09 G15NL327 G15NL332 G15NL341 G15NL304 G15NL356 G17NL223 G17NL188 G17NL176 G17NL197 G17NL219 G17NL236 G17NL242 G18NL243 G15NL07 G15NL19 G15NL23 G15NL30 G15NL326 G15NL329 G15NL339 G15NL347 G15NL353 G16NL37 G16NL39 G16NL47 G16NL49 G16NL104 G16NL134 G17NL160 G17NL185 G17NL186 G17NL195 G17NL201 G17NL232 G18NL254 G18NL258 G18NL265 G17NL235 G18NL244 G18NL245 G18NL251 G18NL278 G18NL676 G15NL01 G15NL15 G15NL318 G15NL323 G16NL40 G16NL56 G16NL64 G16NL88 G16NL122 G16NL131 G16NL151 G16NL148 G17NL167 G17NL168 G17NL203 G17NL208 G17NL211 G17NL226 G17NL241 G18NL252 G18NL267 G18NL277 G15NL10 G15NL314 G15NL301 G15NL340 G15NL285 G16NL41 G16NL90 G16NL108 G16NL112 G16NL138 G16NL143 G17NL217 G17NL229 G18NL259 G15NL08 G15NL18 G15NL22 G15NL319 G15NL302 G15NL355 G15NL305 G15NL289 G15NL296 G16NL55 G16NL68 G16NL70 G16NL101 G16NL102 G16NL115 G16NL120 G16NL155 G17NL164 G17NL173 G18NL273 G18NL275 G15NL365 G15NL303 G15NL338 G15NL352 G15NL286 G16NL44 G16NL127 G16NL137 G16NL141 G16NL144 G16NL152 G17NL158 G17NL177 G17NL190 G18NL260 G18NL269 G15NL06 G15NL25 G15NL33 G15NL328 G15NL333 G15NL292 G15NL293 G16NL58 G16NL79 G16NL118 G16NL121 G16NL135 G16NL117 G16NL129 G17NL162 G17NL216 G18NL250 G18NL271 G18NL279 G15NL02 G15NL20 G15NL35 G15NL363 G15NL316 G15NL324 G15NL337 G15NL349 G15NL284 G16NL45 G16NL87 G16NL103 G16NL145 G17NL161 G17NL172 G17NL183 G17NL205 G17NL238 G17NL239 G15NL17 G15NL34 G15NL369 G15NL307 G15NL317 G15NL300 G15NL321 G15NL325 G15NL359 G15NL287 G16NL74 G16NL80 G16NL128 G16NL147 G17NL165 G17NL204 G16NL113
2	G16NL690 G16NL681 G16NL765 G16NL773 G16NL807 G16NL870 G16NL871 G16NL886 G16NL887 G16NL920 G17NL438 G17NL637 G17NL663 G17NL456 G17NL466 G17NL471 G17NL477 G17NL500 G17NL537 G17NL550 G17NL556 G17NL560 G17NL584 G17NL586 G17NL597 G18NL668 G18NL671 G16NL713 G16NL778 G16NL785 G16NL787 G16NL791 G16NL811 G16NL820 G16NL821 G16NL826 G16NL828 G16NL849 G16NL856 G16NL857 G16NL865 G16NL889 G17NL377 G17NL415 G17NL417 G17NL425 G17NL653 G17NL488 G17NL595 G18NL665 G18NL669 G18NL674 G16NL686 G16NL714 G16NL719 G16NL721 G16NL722 G16NL756 G16NL789 G16NL815 G16NL914 G16NL824 G16NL881 G16NL901 G16NL918 G17NL625 G17NL395 G17NL412 G17NL436 G17NL631 G17NL644 G17NL655 G17NL442 G17NL446 G17NL497 G17NL509 G17NL513 G17NL529 G18NL673 G16NL678 G16NL697 G16NL701 G16NL704 G16NL706 G16NL729 G16NL739 G16NL744 G16NL784 G16NL790 G16NL819 G16NL825 G16NL840 G16NL845 G16NL846 G16NL902 G16NL903 G17NL374 G17NL388 G17NL399 G17NL408 G17NL636 G17NL453 G17NL533 G17NL540 G17NL559 G17NL567 G17NL602 G17NL623 G18NL670 G16NL698 G16NL758 G16NL796 G16NL799 G16NL812 G16NL913 G16NL834 G16NL839 G16NL859 G16NL895 G17NL373 G17NL378 G17NL381 G17NL414 G17NL418 G17NL420 G17NL439 G17NL639 G17NL646 G17NL648 G17NL448 G17NL462 G17NL495 G17NL502 G17NL510 G17NL522 G17NL525 G17NL563 G17NL568 G17NL578 G17NL591 G16NL694 G16NL700 G16NL702 G16NL708 G16NL709 G16NL725 G16NL727 G16NL742 G16NL750 G16NL793 G16NL800 G16NL801 G16NL802 G16NL843 G16NL858 G16NL861 G16NL862 G16NL872 G16NL908 G16NL916 G17NL375 G17NL396

Table S 2.5 continued

Cluster	Genotypes
	G17NL422 G17NL435 G17NL645 G17NL450 G17NL451 G17NL460 G17NL464 G17NL479 G17NL511 G17NL515 G17NL574 G17NL575 G17NL581 G17NL587 G17NL609 G16NL715 G16NL716 G16NL723 G16NL734 G16NL735 G16NL738 G16NL745 G16NL751 G16NL759 G16NL766 G16NL776 G16NL780 G16NL797 G16NL806 G16NL836 G16NL853 G16NL884 G16NL888 G16NL891 G16NL893 G16NL898 G17NL384 G17NL391 G17NL392 G17NL401 G17NL402 G17NL428 G17NL437 G17NL630 G17NL651 G17NL443 G17NL445 G17NL457 G17NL467 G17NL489 G17NL503 G17NL514 G17NL527 G17NL561 G17NL565 G17NL580 G17NL588 G17NL620 G17NL624 G18NL672 G17NL657 G17NL664 G17NL496 G17NL508 G17NL554 G17NL459 G17NL472 G17NL476 G17NL582 G16NL737 G16NL748 G16NL760 G16NL808 G16NL817 G16NL915 G16NL838 G16NL847 G16NL878 G16NL883 G16NL896 G17NL627 G17NL379 G17NL393 G17NL416 G17NL426 G17NL433 G17NL642 G17NL656 G17NL658 G17NL475 G17NL486 G17NL501 G17NL506 G17NL518 G17NL543 G17NL546 G17NL558 G17NL590 G17NL600 G17NL613 G16NL730 G16NL743 G16NL746 G16NL755 G16NL770 G16NL788 G16NL792 G16NL805 G16NL852 G16NL874 G16NL876 G16NL890 G16NL894 G16NL906 G16NL909 G17NL376 G17NL383 G17NL400 G17NL405 G17NL419 G17NL641 G17NL650 G17NL654 G17NL662 G17NL473 G17NL535 G17NL539 G17NL553 G17NL570 G17NL576 G17NL599 G17NL604 G17NL615 G17NL619 G18NL667 G18NL675 G17NL504 G17NL512 G17NL519 G17NL528 G17NL534 G17NL579 G17NL583 G17NL596 G17NL608 G17NL616 G16NL689 G16NL728 G16NL753 G16NL762 G16NL769 G16NL803 G16NL816 G16NL823 G16NL829 G16NL837 G16NL850 G16NL921 G17NL390 G17NL413 G17NL429 G17NL633 G17NL643 G17NL465 G17NL478 G17NL480 G17NL487 G17NL490 G17NL493 G17NL516 G17NL517 G17NL520 G17NL544 G17NL545 G17NL552 G17NL555 G17NL571 G17NL572 G17NL585 G17NL603 G17NL606 G16NL682 G16NL692 G16NL717 G16NL733 G16NL736 G16NL749 G16NL772 G16NL775 G16NL781 G16NL782 G16NL786 G16NL848 G16NL869 G16NL882 G16NL892 G17NL628 G17NL389 G17NL394 G17NL403 G17NL406 G17NL407 G17NL421 G17NL660 G17NL447 G17NL461 G17NL481 G17NL498 G17NL507 G17NL530 G17NL564 G17NL573 G17NL618 G16NL685 G16NL696 G16NL699 G16NL712 G16NL732 G16NL752 G16NL763 G16NL767 G16NL771 G16NL794 G16NL795 G16NL798 G16NL844 G16NL854 G16NL855 G16NL864 G16NL880 G16NL899 G16NL907 G17NL397 G17NL423 G17NL424 G17NL430 G17NL432 G17NL440 G17NL638 G17NL494 G17NL531 G17NL566 G17NL589 G17NL605 G17NL612 G17NL617 G16NL677 G16NL688 G16NL693 G16NL695 G16NL754 G16NL761 G16NL783 G16NL810 G16NL818 G16NL831 G16NL832 G16NL835 G16NL873 G16NL877 G16NL905 G16NL911 G16NL917 G17NL626 G17NL386 G17NL398 G17NL409 G17NL431 G17NL434 G17NL661 G17NL441 G17NL444 G17NL449 G17NL452 G17NL454 G17NL463 G17NL484 G17NL491 G17NL536 G17NL538 G17NL569 G17NL577 G17NL601 G17NL607 G17NL614 G18NL666 G16NL680 G16NL687 G16NL705 G16NL724 G16NL718 G16NL726 G16NL731 G16NL740 G16NL747 G16NL764 G16NL809 G16NL813 G16NL822 G16NL827 G16NL885 G16NL900 G16NL910 G16NL912 G16NL919 G17NL380 G17NL382 G17NL411 G17NL634 G17NL647 G17NL652 G17NL659 G17NL469 G17NL492 G17NL521 G17NL523 G17NL526 G17NL532 G17NL541 G17NL547 G17NL548 G17NL557 G17NL593 G17NL594 G17NL598 G17NL621 G17NL622 G16NL679 G16NL683 G16NL703 G16NL711 G16NL720 G16NL779 G16NL842 G16NL860 G16NL863 G16NL866 G16NL868 G16NL879 G16NL897 G16NL904 G17NL385 G17NL427 G17NL629 G17NL640 G17NL455 G17NL474 G17NL482 G17NL483 G17NL524 G17NL542 G17NL551 G17NL562 G17NL592 G17NL610 G17NL611 G16NL691 G16NL741 G16NL757 G16NL768 G16NL774 G16NL777 G16NL804 G16NL830 G16NL833 G16NL841 G16NL867 G16NL875 G17NL387 G17NL404 G17NL410 G17NL635 G17NL649 G17NL458 G17NL470 G17NL499

Table S 2.6 Contrasting maize lines selected with the nearest and furthest distances among the 182 founder inbred lines based on pairwise genetic comparisons using SNP markers.

Line 1	**Line 2**	**Genetic distance (centimorgans (cM))**
Selection of lines with nearest genetic distances		
16AG16786	16AG16785	0.006
16AG16802	16AG16801	0.013
16AG16791	16AG16793	0.014
16AG16795	16AG16794	0.014
16AG16809	16AG16810	0.014
16AG16790	16AG16791	0.014
16AG16810	16AG16809	0.014
GS-PL17	GS-PL53	0.021
GS-PL40	GS-PL39	0.026
16AG16785	16AG16786	0.04
GL16828OS	16AG16813	0.046
15NN166W	15AG146	0.055
15NN117W	15AG113	0.063
15AG149	15AG150	0.067
15AG109	15AG108	0.071
15AG108	15AG110	0.071
16AG16815	GS-PL26	0.072
16AG16803	16AG16807	0.073
15AG171	GS-PL08	0.075
16AG16783	16AG16784	0.077
15AG152	15AG153	0.078
15AG172	15AG173	0.078
16AG16811	16AG16812	0.079
16AG16789	16AG16790	0.085
GS-PL05	15AG115	0.086
16AG16803	16AG16804	0.087
16AG16796	16AG16810	0.098
15AG127	15AG128	0.104
16AG16816	16AG16815	0.105
15AG117	GS-PL04	0.111
15AG144	15AG152	0.111
16AG179	GS-PL15	0.112
15AG129	15AG130	0.113
16AG16817	GS-PL26	0.114
15AG162	15AG163	0.114
GS-PL07	15AG160	0.123
16AG16797	16AG16799	0.123
15AG143	15AG152	0.124
15AG174	15AG175	0.127
15AG126	15AG132	0.128
15AG140	15AG146	0.131
GS-PL43	GS-PL08	0.134
16AG16808	GS-PL49	0.134
GS-PL65	GS-PL49	0.134
16AG16800	16AG16799	0.136
15AG131	15AG125	0.138
15AG124	15AG126	0.142
GS-PL62	GS-PL09	0.147
15AG133	15AG127	0.147
16AG16798	16AG16803	0.149

Table S 2.6 continued

Line 1	Line 2	Genetic distance (centimorgans (cM))
Selection of lines with the furthest genetic distances		
15AG112	GS-PL44	0.425
15AG169	GS-PL44	0.425
GS-PL28	GS-PL44	0.425
16AG16791	GS-PL44	0.425
GS-PL07	GS-PL44	0.425
GS-PL47	GS-PL44	0.425
GS-PL44	15AG125	0.425
GS-PL44	GS-PL06	0.425
GS-PL44	15AG109	0.425
GS-PL44	16AG16812	0.425
GS-PL44	GS-PL69	0.425
GS-PL44	15AG153	0.425
GS-PL44	15AG146	0.425
GS-PL22	GS-PL44	0.426
16AG16792	GS-PL44	0.426
15AG144	GS-PL44	0.426
15AG140	GS-PL44	0.426
GS-PL59	GS-PL44	0.426
16AG178	GS-PL44	0.426
15AG133	GS-PL44	0.426
15AG124	GS-PL44	0.426
GS-PL44	15AG143	0.426
GS-PL44	15AG114	0.426
GS-PL44	GS-PL50	0.426
GS-PL14	GS-PL44	0.427
15AG128	GS-PL44	0.427
15AG150	GS-PL44	0.427
16AG16789	GS-PL44	0.427
15AG148	GS-PL44	0.427
GS-PL44	15AG132	0.427
GS-PL44	15AG131	0.427
GS-PL44	GS-PL58	0.427
GS-PL44	GS-PL16	0.427
GS-PL44	15AG108	0.427
GS-PL44	16AG16816	0.427
GS-PL44	15AG123	0.427
GS-PL37	GS-PL44	0.428
15AG177	GS-PL44	0.428
16AG16788	GS-PL44	0.428
15AG162	GS-PL44	0.428
15AG155	GS-PL44	0.428
16AG16790	GS-PL44	0.428
15AG115	GS-PL44	0.428
GS-PL01	GS-PL44	0.428
GS-PL44	GS-PL24	0.428
GS-PL44	15AG174	0.428
GS-PL71	GS-PL44	0.429
15AG134	GS-PL44	0.429
15AG126	GS-PL44	0.43
GS-PL54	GS-PL44	0.431
GS-PL44	15AG149	0.431
15AG152	GS-PL44	0.432
GS-PL33	GS-PL44	0.435

Table S 2.7 Contrasting maize lines selected with the nearest and furthest distances among the 866 derived inbred lines based on pairwise genetic comparisons using SNP markers.

Line 1	**Line 2**	**Genetic distance (centimorgans (cM))**
Selection of lines with nearest genetic distances		
G17NL211	G17NL210	0.004
G17NL473	G17NL472	0.005
G17NL194	G16NL910	0.008
G16NL804	G16NL806	0.008
G16NL854	G16NL853	0.009
G16NL853	G16NL855	0.009
G17NL602	G17NL603	0.011
G16NL895	G16NL896	0.011
G16NL919	G16NL920	0.015
G16NL805	G16NL806	0.015
G16NL894	G16NL895	0.016
G18NL261	G18NL260	0.017
G17NL626	G17NL625	0.019
G17NL159	G17NL557	0.019
G17NL557	G17NL159	0.019
G17NL215	G17NL216	0.02
G17NL635	G17NL636	0.021
G16NL759	G16NL758	0.021
G16NL880	G16NL879	0.021
G17NL201	G17NL200	0.022
G16NL891	G16NL893	0.023
G16NL58	G16NL792	0.023
G16NL824	G16NL825	0.023
G16NL709	G16NL708	0.024
G16NL748	G16NL747	0.024
G16NL799	G16NL800	0.025
G16NL881	G16NL879	0.025
G16NL899	G16NL900	0.025
G17NL409	G17NL408	0.026
G17NL504	G17NL505	0.026
G17NL480	G17NL481	0.027
G16NL148	G16NL149	0.027
G16NL731	G16NL730	0.029
G16NL730	G16NL732	0.029
G16NL115	G18NL279	0.03
G18NL266	G18NL267	0.03
G16NL768	G16NL769	0.03
G16NL700	G16NL701	0.03
G16NL778	G16NL779	0.03
G16NL99	G16NL100	0.032

Table S 2.7 continued

Line 1	Line 2	Genetic distance (centimorgans (cM))
G16NL780	G16NL781	0.034
G16NL892	G16NL893	0.034
G16NL723	G16NL724	0.036
G16NL863	G16NL864	0.036
G16NL817	G16NL818	0.036
G16NL842	G16NL843	0.037
G16NL874	G16NL875	0.037
G16NL915	G16NL834	0.037
G17NL241	G17NL242	0.038
Selection of lines with the furthest genetic distances		
G15NL342	G15NL302	0.267
G15NL326	G15NL321	0.267
G15NL298	G15NL336	0.267
G15NL344	G15NL301	0.268
G16NL54	G15NL316	0.27
G16NL62	G16NL789	0.271
G15NL285	G16NL65	0.271
G16NL691	G15NL298	0.271
G15NL294	G16NL682	0.273
G15NL288	G15NL298	0.273
G15NL293	G15NL295	0.273
G15NL360	G15NL307	0.274
G15NL08	G15NL307	0.276
G15NL348	G15NL360	0.277
G16NL912	G16NL899	0.277
G16NL886	G15NL31	0.278
G15NL312	G15NL306	0.279
G15NL325	G15NL324	0.28
G16NL144	G16NL86	0.28
G15NL332	G15NL328	0.281
G15NL333	G15NL354	0.282
G15NL317	G15NL306	0.283
G15NL300	G17NL237	0.284
G15NL323	G15NL312	0.287
G15NL289	G15NL285	0.288
G15NL286	G18NL262	0.288
G15NL365	G15NL341	0.289
G15NL287	G15NL297	0.291
G15NL318	G15NL306	0.291
G15NL310	G15NL313	0.293
G15NL319	G15NL369	0.293
G15NL334	G15NL291	0.294
G15NL355	G15NL301	0.298
G15NL292	G15NL284	0.299
G15NL327	G15NL353	0.301
G15NL303	G15NL357	0.303
G15NL349	G15NL310	0.31
G15NL337	G15NL312	0.336

CHAPTER THREE

Genome-wide Prediction of Yield and Component Traits in Maize Using Qualitative and Quantitative Phenotypic Traits and Genomic Best Linear Unbiased Prediction Model

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Abstract

New and pipeline hybrid breeding in maize and related crops requires genetically superior and contrasting inbred lines for yield, component traits and product profiles. The genomic best linear unbiased prediction (GBLUP) model is widely used to predict the selection response of qualitative and quantitative phenotypic traits. Genome-wide prediction of yield and component traits was conducted using qualitative and quantitative phenotypic traits and single nucleotide polymorphism (SNP) markers based on the additive-dominant genomic best linear unbiased prediction (GBLUP) model to guide inbred line selection and hybrid breeding. Genomic estimated breeding values (GEBVs) and genomic estimated genetic values (GEGVs) were computed for yield, yield components and major foliar diseases using GBLUP. Genetically diverse 1102 inbred lines from two heterotic groups [N3 (group 1) and SC (group 2)] were genotyped using high-density SNP markers. Phenotypic data were collected based on field trials using the following single-cross hybrids (SCH) developed by crossing the 1102 inbred lines to 4 inbred line testers: 684 SCHs evaluated at five locations in 2018/19, 760 at four locations (2019/20), 646 at four locations (2020/21), and 740 at four locations (2021/22) summer seasons in Zimbabwe. The study selected 20 high-performing and contrasting inbred lines with the highest GEBVs and GEGVs each from the two heterotic groups, for genetic advancement, combining ability tests and commercial hybrid development. In addition, 20 high-performing candidate SCHs with high GEGVs were identified for three-way hybrid development, variety registration and commercialization. Integrating genomic prediction into conventional maize breeding programs is essential to identify the genetic identity of candidate inbred lines and develop high-yielding hybrids with market-preferred product profiles.

Keywords: *genome-wide prediction, genotyping, heterotic groups, hybrid breeding, inbred lines, Zea mays*

3.1 Introduction

Genetically superior and contrasting inbred lines are the powerhouse of new and pipeline hybrid breeding programs in maize (*Zea mays* L., 2n = 2x =20) and related crops (Chen et al., 2021). However, developing these inbred lines requires broader and larger genetic pools, targeted crosses, continuous selfing, and combining ability tests and selection to exploit hybrid vigour. Various mating designs are used to determine the combining ability effects of homozygous inbred lines and experimental hybrids, including the line-by-tester genetic design (Hallauer et al., 2010). Conventional breeding employs multiple crosses to identify the best lines and hybrids, which is time-consuming and expensive. This approach may limit the sample size of hybrid combinations and the response to selection of the best combiner lines and hybrids (Schrag et al., 2010). Hence, complementary genetic analysis is vital, using effective molecular markers and genetic models to discern the genetic identity of inbred lines and predict novel cross combinations based on yield, component traits, and major disease reactions.

Molecular markers have revolutionized maize breeding by enabling genome-wide selection for complex traits. This allows for the prediction and leveraging of large and small QTLs associated with markers (Albrecht et al., 2011; Clark et al., 2013; Crossa et al., 2013; Beyene et al., 2019). Recently identified marker alleles are tightly linked to genes controlling these traits and exhibit strong linkage disequilibrium. As a result, it is highly likely that desirable genes will be inherited alongside these markers. Progeny lines developed from desirable parents will inherit a combination of marker alleles that are similar to, or identical with, one of the two alleles present in each parent. The exact combination is determined by allelic segregation during meiosis. These markers can then be used as diagnostic tools to accelerate the development of high-performing maize varieties with improved productivity and quality. This builds on the foundation of traditional phenotypic selection (Semagn et al., 2006; Collard & Mackill, 2007; Misztal et al., 2009).

Most economically significant traits, such as grain yield and drought tolerance, are polygenic and are affected by several minor genes and the genotype x environment interaction (GEI) effect. To select polygenic traits and limit the GEI effect, genomic selection (GS) is used to optimize the selection of quantitative traits. GS uses genome-wide molecular markers and historical phenotypic data to predict the performance of genetically related individuals (Meuwissen et al., 2001; Heslot et al., 2012). Single nucleotide polymorphism (SNPs) is the most widely used marker system for GS in maize. SNPs are widely present in the genome and vary across genotypes (Rafalski, 2002). SNPs are the most preferred markers in crop genetics and plant breeding programs because of their low cost, high-genomic abundance, locus specificity, co-dominant inheritance, amenability to high-throughput genotyping, and relatively low genotyping error rates (Rafalski, 2002; Schlötterer, 2004; Bui et al., 2017). There are several genotyping platforms available for SNP analysis (Rafalski, 2002), such as Affymetrix, Midseq, TaqMan, rhAmp, Kompetitive Allele-Specific (KASP), Illumina, MassARRAY, Iplex, Target SNP-seq and SNPstream platforms. These platforms offer differences in terms of their assay design flexibility, assay design success rates, allele call rate and quality, ease of experiment run and cost per sample (Svidnicki et al., 2015; Ayalew et al., 2019; Zhang et al., 2020).

The genomic best linear unbiased prediction (GBLUP) model is widely used to predict the selection response of qualitative and quantitative phenotypic traits, genome-wide association studies, prediction of genomic estimated breeding values (GEBVs) and genomic estimated genetic values (GEGVs), identification of causal variants and genes, investigation of genetic architecture and trait relationships, and development of precision breeding strategies and personalized breeding plans. The model uses a genomic relationship matrix (G matrix) that describes genetic relationships between individuals, calculated from genotypes using SNPs, to estimate genetic variance and covariance (Habier et al., 2007; Van Raden (2008). The matrix defines the covariance between individuals based on observed similarity at the genomic level rather than on expected similarity based on pedigree information of morpho-agronomic traits. GBLUP's computational efficiency enables accurate predictions of genetic values by integrating genomic data, capturing subtle effects, and accounting for complex traits, leading to informed breeding decisions and accelerated genetic progress (Mackay et al., 2015; Bernardo, 2021). GBLUP has been applied to predict genotype performance using phenotypic data, (including traits such as plant height, ear length, lodging tolerance, ear diameter, husk cover, number of rows per cob, number of kernels per cob, and grain yield), in addition to estimating variance components and genomic heritability of key economic traits in maize. To enhance its predictive capabilities, high-quality phenotypic observations are essential for training robust models (Crossa et al., 2013; Cooper et al., 2016; Liu et al., 2018; Yang et al., 2020).

Genomic selection (GS) is achieved with a model for computing GEBVs and GEGVs by using a training population of individuals who have undergone rigorous genotyping and phenotyping (Habier et al., 2013;

Hickey et al., 2014; Bassi et al., 2015; Crossa et al., 2017b; Varshney et al., 2017). Using the GBLUP model, GEBVs and GEGVs can be computed for new individuals from a candidate population to select genetically unique candidates and develop new hybrid populations. The GBLUP model captures the total additive genetic variance through genome-wide marker coverage to compute GEBV estimates (Schrag et al., 2008; Albrecht et al., 2011; Rutkoski et al., 2011; Windhausen et al., 2012; Riedelsheimer et al., 2013). Non-additive genetic variances (e.g. epistatic and dominance genetic effects) can be included through genome-wide marker coverage by extending the basic GBLUP model to calculate the GEGVs of individuals (Jiang & Reif, 2015; Martini et al., 2016). Genes conditioning the trait of interest are assumed to be in linkage disequilibrium (LD) with a subset of the markers, enabling the capture of genetic variation associated with the trait. Breeding and genetic values of individuals can be predicted using the GBLUP model without phenotypic data. Predictions using the GBLUP model is in contrast to classical marker-assisted selection (MAS), which uses only a small number of markers assumed to have a significant impact on the underlying trait of interest (Collard et al., 2005; Semagn et al., 2006; Collard & Mackill, 2007; Misztal et al., 2009).

The slow pace of developing high-value inbred lines and hybrids and the high phenotyping costs remain challenging in most breeding programs. To enhance the turnaround of lines and hybrids development and to reduce phenotyping costs, the Seed-Co breeding programme adopted genomic selection to improve its breeding efficiency. Based on genomic-enabled prediction results, GS is promising in germplasm enhancement that could accelerate the flow of yield-influencing genes from diverse sources. With the current pipeline of the conventional breeding method, it takes five to six years to develop experimental hybrids and undertake multi-location yield trials and variety replacement. GS is vital to predicting advanced-generation performance based on early-generation genotyping. This would have multiple advantages, including reduced breeding time and cycle, increased number of inbred lines and crosses (by providing more accurate breeding value predictions, identifying more potential parents and superior alleles), and increased selection intensity. The GBLUP model predicts GEBVs and GEGVs, leveraging genotypic data and optional phenotypic records, to identify genetically distinct inbred lines and estimate their breeding potential. This will enable new and targeted hybrid development using contrasting inbred lines. Genome-wide genetic information and prediction enables precision and speed breeding. Therefore, the objective of the study was to conduct genome-wide prediction of yield and component traits using qualitative and quantitative phenotypic traits and single nucleotide polymorphism (SNP) markers based on the additive-dominant genomic best linear unbiased predictions model to compute genomic estimated breeding values and genomic estimated genetic values to guide inbred line development and hybrid breeding.

3.2 Materials and methods

3.2.1 Plant materials

The study used 1102 inbred lines sampled from two heterotic backgrounds [N3 (group 1) and SC (group 2)]. The inbred lines used in the study are summarised in Supplementary Table 3.1 (Table S 3.1). The lines were sourced from Seed-Co Ltd and the International Maize and Wheat Improvement Centre (CIMMYT) germplasm pool in Zimbabwe. Seed-Co Ltd developed the lines for pipeline hybrid breeding aiming for high grain yield, yield components and tolerance to biotic and abiotic stress.

3.2.2 Development of single cross hybrids

The study used a line-by-tester mating design to develop single cross hybrids. The inbred lines were crossed with four inbred line testers with good combining ability for grain yield and yield components and tolerance to biotic and abiotic stress. Testers from the opposite heterotic group were used as males and crossed with the lines used as females from the other heterotic group. Testers from the N3 heterotic group were crossed to lines from the SC heterotic group, and testers from the SC heterotic group were crossed to lines from the N3 heterotic group to maximize heterosis.

3.2.3 Study sites

The single cross hybrids (hereafter referred to as crosses) were subjected to field trials at seven locations in Zimbabwe between 2018 and 2022. The description of the study sites is provided in Table 3.1.

Table 3.1 Description of the seven study sites used to evaluate the crosses in Zimbabwe between 2018 and 2022.

Site name	Abbreviation	Altitude (masl)	Latitude	Longitude	Rainfall (mm)	Maximum temperature (°C)	Minimum temperature (°C)
Rattray Arnold Research Station	RARS	1360	17°14'S	31°14'E	865	27.4	15.2
Kadoma Research Centre	KRC	1149	18°16’S	29°50’E	700	28	20
Stapleford	STAP	1476	17°43’S	30°52’E	800	28	15
University of Zimbabwe Farm	UZ Farm	1468	17°80’S	31°05’E	820	28	12
Chakari	CHAK	1099	18°04’S	29°51’E	800	27	12
Gwenhoro	GWEN	1262	19°43’S	29°51’E	830	28	15
Mvurwi	MV	1493	17°03’S	30°51’E	880	27	15

masl=metres above sea level

3.2.4 Experimental design and trial management

Feld evaluations were conducted at seven sites (Table 3.1) in the summer season spanning from November to April in the 2018/19, 2019/20, 2020/21 and 2021/22 seasons. The study evaluated 684 single cross hybrids at five locations (RARS, KRC, STAP, UZ FARM, MV) in 2018/19, 760 at four locations (RARS, KRC, STAP, GWEN) (2019/20), 646 at four locations (RARS, KRC, STAP, CHAK) (2020/21), and 740 at four locations (RARS, KRC, STAP, CHAK) (2021/22). The crosses evaluated in each season were divided into 42 entry trials consisting of 38 single cross hybrid trial entries and 4 single cross hybrid commercial check varieties. In the 2018/19 season, 18 trials were evaluated, 20 in 2019/20 and 17 in 2020/21. In the 2021/22 season, each trial comprised 37 single cross hybrid trial entries and 5 single cross hybrid check varieties, making the number of trials evaluated that season at 20. The field trials were laid out in a 6 x 7 alpha lattice design with two replications at each site. The experimental unit consisted of two rows measuring 6 m long with inter-row and intra-row spacing of 0.75 m and 0.25 m, respectively. Three seeds were sown per hill and thinned to one plant per hill one week after emergence to achieve an approximate plant population of 53 333 plants ha $^{-1}$. Maize fertilizer containing Nitrogen, Phosphorous and Potassium in a ratio of 7:14:7, in that order, was used as a basal application at a rate of 426 kg ha^{-1} at all the testing sites. Ammonium nitrate (34.5 % N) was split applied as top-dressing fertiliser at a rate of 426 kg ha^{-1} at each site. Half of this amount was applied at four weeks after planting and the remainder at eight weeks after planting. The trials were kept weed-free using chemical and mechanical weed control. A blend of Alachlor (3.5 litres ha $^{-1}$), Atrazine (2.5 litres ha^{-1}) and Glyphosate (2.5 litres ha $^{-1}$) was applied as pre-emergence herbicide. Hand weeding was done to remove any weed flashes which appeared thereafter. Scouting for insect pests such as maize stalk borer (*Busseola fusca*) and fall armyworm (*Spodoptera frugiperda*) was done soon after crop emergence and continued during the growth of the crops. Stalk borer was controlled using dipterex granules, and application was done four weeks after the crop emergency. The granules were applied in each plant's leaf whorl or funnel at a rate of 2 kg ha^{-1}. Alternate Super Dash and Blast Super sprays were applied as full cover at 1 litre ha^{-1} to control the fall armyworm. The experiments were conducted under rain-fed conditions at all sites. Supplementary irrigation was provided during dry periods as required.

3.2.5 Phenotyping

Data were collected on grain yield and yield-related traits (Table 3.2). Disease assessments were made for infections caused by the *Maize streak virus* (MSV), grey leaf spot (GLS) [*Cercospora zeae maydis*], *phaesophaeria* leaf spot (PLS) [*Phaesophaeria maydis*] and rust (RST) [*Puccinia polysora*]. The severity of the above diseases was rated using a scale of 1-9 (Table 3.3) (Gulzar et al., 2018). Hybrids showing disease scores of <3.0 were considered resistant, 3.1-5.0 moderately resistant, 5.1-7.0 moderately susceptible and >7.1 susceptible (Gulzar et al., 2018). Disease rating was done a month after tasselling, while the leaves were green. The disease reactions were based on natural infestations in the field.

Table 3.2 List and description of phenotypic traits recorded in the study.

Traits	Abbreviation	Description (units)
Agronomic		
Days-to-mid-pollen	AD	Measured as the number of days from planting date to 50% pollen shed (days).
Days-to-mid-silking	SD	Measured as the number of days from planting date to 50% silk emergency (days).
Anthesis-silking-interval	ASI	Calculated as the difference between days-to-mid-pollen to days-to-mid-silking (days).
Plant height	PH	Determined by randomly selecting and tagging 10 plants from a plot (6 m^2) and measured as the distance between the base of the plant and where the tassel starts to branch (expressed in centimetre)
Ear height	EH	Determined from 10 randomly selected and tagged plants by measuring the distance from the base of the plant to the node that bears the upper most ear using a measuring tape (expressed in centimetre).
Root lodging tolerance	RL	Calculated as the number of plants leaning more than 45º from the upright position expressed as a percentage of total plants (NP) (%).
Stem lodging tolerance	SL	Calculated as the number of plant stems broken below the ear expressed as a percentage of the number of plants (%).
Total lodging tolerance	TL	Determined by adding the number of plants that lodged as a result of root and stem lodging (%).
Number of plants per $6m^2$	NP	Determined by recording the number of plants per plot (6 m^2) at harvest.
Husk cover number	HCno	Calculated as the number of ears with open or loose husks.
Husk cover	HC	Calculated as the number of ears with open or loose husks as a percentage of plant density (%).
Ears harvested per $6m^2$	EHP	Determined by recording the number of ears counted at harvest per $6m^2$.
Ears per plant	EPP	Calculated as a ratio of ear count to the number of plants per $6m^2$.
Grain yield	GY	Determined by adjusting grain weight to 12.5% moisture content and converting to tonnes per hectare.
Major diseases		
Maize streak virus reaction	MSV	Determined by carrying a visual estimation of maize streak virus severity on the plants. Severity rated using a scale of 1-9.
Gray leaf spot reaction	GLS	Determined by carrying a visual estimation of gray leaf spot severity on the plants. Severity rated using a scale of 1-9.
Phaesopharia leaf spot reaction	PLS	Determined by carrying a visual estimation of *phaesopharia* leaf spot severity on the plants. Severity rated using a scale of 1-9.
Rust reaction	RST	Determined by carrying a visual estimation of rust severity on the plants. Severity rated using a scale of 1-9.

Grain yield expressed in t/ha $^{-1}$ was obtained from grain weight per plot adjusted to 12.5% grain moisture following CIMMYT (1999).

$$\mathrm{GY} = \left(\frac{\mathrm{GW}}{\mathrm{NP}}\right) * 10 * \left(\frac{100 - \mathrm{MO}}{87.5}\right)$$

Where;

GY= Grain yield (kg/ha)

GW=Grain weight at harvest (kg/$6m^2$)

MO= Moisture content (%) of grains at harvest.

87.5= Standard dry matter of grain at 12.5 % as required by maize grain market authorities in Zimbabwe and South Africa

NP= Net plot area (number of rows x intra row spacing x number of stations x inter row spacing).

Table 3.3 Foliar disease rating scale, description and severity in maize (Gulzar et al., 2018).

Rating scale	**Description of infection**	**Disease severity (%)**
1	None to very slight disease infection.	≤10%
2	Slight infection with few lesions on two lower leaves.	10.1-20
3	Light infection with moderate number of disease lesions spread on four lower leaves.	20.1-30
4	Light infection with moderate number of disease lesions scattered abundantly on lower leaves and a few lesions on middle leaves below the ear.	30.1-40
5	Moderate infection with abundant number of lesions on lower leaves moderate and number of lesions scattered on middle leaves below the ear.	40.1-50
6	Heavy infection with lesions scattered abundantly on lower leaves and moderate disease infection on middle leaves.	50.1-60
7	Heavy infection with lesions abundantly scattered on lower and middle leaves and moderate number of lesions on two to four leaves above the ear.	60.1-70
8	Very heavy infection with lesions abundantly scattered on lower leaves and middle leaves, spreading up to the flag leaf.	70.1-80
9	Very heavy infection with disease lesions abundantly scattered on almost all the leaves with the plant prematurely dried and killed.	>80%

3.2.6 Data analysis of phenotypic traits and disease severity

Single site phenotypic data analysis and mean separation was performed following Dabholkar (1999):

$$Y_{ilk} = \mu + h_i + r_k + b_l\ (r_k) + e_{ilk}.$$

Where, Y_{ilk} = observed response; μ = overall trial mean; h_i = effect of the i^{th} hybrid; r_k = effect of the k^{th} replication; $b_l\ (r_k)$ = effect of the l^{th} block nested in the k^{th} replication and e_{ilk} = random error

Across site phenotypic data analysis and mean separation was performed based on the formula given by Singh and Chaudhary (1979):

$$Y_{ijkl} = \mu + G_j + E_i + G_j * E_i + Ri + Bl(Ei) + E_i\ (rk)\ (b_l) + e_{ijkl}$$

Where: Y_{ijkl} is the observed response; μ is the grand mean (fixed effect); G_j is the effect of the j^{th} genotype (fixed effect); E_i is the effect of the i^{th} environment (fixed effect); G_j*E_i is the genotype x environment interaction (fixed effect); R_i is the effect of the i^{th} replication (fixed effect); $B_l(E_i)$ is the effect of the l^{th} block within the i^{th} environment (fixed effect); $E_i(r_k)(b_l)$ is the error associated with the k^{th} replication in the l^{th} block in the i^{th} environment (random effect), it captures the variation within environments, blocks, and replications; e_{ijkl} is the random error (random effect), it accounts for the residual variation.

3.2.7 Broad-sense heritability

The broad sense heritability of assessed traits across locations was calculated as described by Hallauer and Miranda (1988):

$$\mathrm{H}^2 = \left[\frac{\sigma^2 g}{\sigma^2 p}\right] \mathrm{X}\ 100$$

Where,

$$\sigma^2 p = \frac{\sigma^2}{re} + \frac{\sigma^2 ge}{e} + \sigma^2 g$$

H^2=broad sense heritability; $\sigma^2 g$ = genotypic variance; σ^2= environment variance; $\sigma^2 ge$= genotype by environment interaction variance; r= number of replications; e= number of sites.

3.2.8 DNA extraction and genotyping

DNA extraction and single nucleotide polymorphism (SNP) testing were carried out by the Limagrain Laboratory (France) following their approved procedures and operational guidelines (www.limagrain.com). The lines for genotyping were divided into two sets. Set 1 comprised of 223 lines and set 2 comprised of 879 lines. Set 1 lines were mainly old elite lines that had been in the Seed-Co germplasm pool for a long time. Set 2 was made up of newly generated lines. Genotyping was done on two platforms: Affymetrix (Set 1) with 36,029 SNP KASP markers and Midseq (Set 2) with 1,484 SNP markers. The genotypic data from both platforms were merged into a unified genotype matrix. Missing genotypes were filled in using a reference panel. Quality control measures were implemented to filter out low-quality SNPs, standardize SNP naming and ordering, and eliminate duplicates or redundant markers. The SNPs from both platforms were aligned to a common reference genome or map. The Kompetitive Allele-Specific PCR (KASP) system tailored DNA extraction method was used to extract genomic DNA from maize kernels. Two kernels were sampled from each inbred line, with positive controls in the first six wells of a 96-well plate and test samples in the remaining 90 wells. The purity and concentration of DNA were tested using a Nanodrop (Nanovue Plus), and the DNA was diluted tenfold with concentrations ranging from 81 to 188ug/l. The DNA was dried before being placed on 384 PCR plates. Pheraster SNP was used to scan the data, and Klustercaller genotyping software was used to process it. Finally, the integrated genotypic data was prepared for genomic prediction.

3.2.9 Prediction of genomic estimated breeding values (GEBVs) and genomic estimated genotypic values (GEGVs)

3.2.9.1 Genomic Prediction Models and Methods

The genomic best linear unbiased prediction model (GBLUP) was used to predict the GEBVs and GEGVs for the lines and hybrids, respectively.

3.2.9.2 Genomic Best Linear Unbiased Predictor (GBLUP) model

The predictive model used for the study was the Genomic Best Linear Unbiased Predictor (GBLUP) (Hayes et al., 2009), with a genomic relationship matrix computed according to VanRaden (2008) using the following method:

$$G = Z * Z' / (\text{number of markers}),$$

Where: G = genomic relationship matrix, Z = matrix of standardized marker genotypes (0, 1, or -1 for each marker), Z' = transpose of matrix Z and number of markers = total number of markers used.

The GBLUP method is the most used parametric method and is vital in capturing additive interactions. The method generates a genomic association matrix using imputed or non-imputed marker data. Genomic estimated breeding and genomic values were calculated for all individuals, and accordingly, the top individuals were selected. The GBLUP model comprises the additive genetic effect of individuals whose variation structure is determined by the kinship matrix created using all SNPs. The GBLUP model is a linear mixed model that accounts for the genetic relationships between individuals and the effects of markers on the phenotype, the model can be written as:

$$\boldsymbol{y = Xb + Zg + e}$$

where: **y** is the vector of phenotypic values (e.g., grain yield, plant height, ear height, number of plants per 6m^2 etc.), **X** is the design matrix for fixed effects (e.g., environment, trial, etc.), **b** is the vector of fixed effects, **Z** is the design matrix for random genetic effects, **g** is the vector of random genetic effects (breeding values) (Note that the vector *g* contains individuals with phenotypic data but can be extended to individuals with no phenotypes. The first group is then referred to as the training or reference population, whereas the latter is the test population or a set of individuals to be predicted) and **e** is the vector of residuals (errors). The random genetic effects (g) are assumed to follow a multivariate normal distribution with a covariance structure defined by the genomic relationship matrix (G):

g ~ N (0, Gσ^2_g)

Where: G is the genomic relationship matrix (GRM) calculated from the SNP markers and σ^2_g is the genetic variance component. The variance components were estimated using the restricted maximum likelihood (REML) method. The GBLUP model used the following formula to predict breeding values (g):

g = (Z^T Z + λI) ^-1 Z^T (y - Xb)

where: **Z^T** is the transpose of the design matrix Z, **λ** is the regularization parameter (related to the variance of the residuals), **I** is the identity matrix and **y - Xb** is the vector of adjusted phenotypes (corrected for fixed effects).

For marker data, the design matrix Z was constructed as follows: **Z** is a matrix of dimension **n x m,** where **n** is the number of individuals and **m** is the number of markers. Each element **Z_ij** represents the genotype of individual **i** at marker **j** (e.g., 0, 1, or 2 for homozygous reference, heterozygous, or homozygous alternative, respectively).

The GEGVs of advanced lines were predicted by incorporating non-additive (i.e., dominance and epistatic) genetic effects into the GBLUP model. From a computational point of view, the most efficient way to include non-additive interactions in genomic selection models is to define appropriate covariance matrices between individual effects in the same way that the standard GBLUP model uses the genomic relationship matrix. In this case, considering the interactive nature of the genetic effects. Simple variations were made to the basic GBLUP model to include epistatic and dominant genetic effects, respectively, as described below;

1. The extended GBLUP model (EGBLUP), described by Jiang & Reif (2015) and Martini et al. (2016), is an extension of the classic GBLUP model designed to include epistasis. The model is described below;

$$y = Xb + Ma + Qaa + e$$

Where y is the vector of phenotypes, X is the design matrix relating the fixed effects to each individual, b is the vector of fixed effects, M is an $n \times p$ matrix of marker profiles, a is the p dimensional vector of additive effects for all markers, a ~ $N(0, I\sigma^2 a)$, Q is an $n \times p(p\text{-}1) \times 2$ dimensional matrix whose columns are products of two distinct columns in M. aa is the vector of epistatic effects of markers, $aa \sim N(o, I\sigma^2 aa)$ and e is the vector of normal deviates with variance $\sigma^2 e$.

2. The variation to the standard GBLUP model to include additive and dominance genetic effects is termed the GBLUP-D model and is expressed as follows;

$$y = Xb + Za + Zd + e$$

Where y is the vector of phenotypes, X and Z are incident matrices for the fixed effects, additive and dominance genetic effects, respectively, b is the vector of fixed effects, a and d are vectors of additive and dominance genetic effects, and e is the vector of residuals.

3.2.10 Cross validation

Cross-validation was employed to evaluate the performance of the GBLUP model by dividing the data into training and testing sets. This approach assessed the accuracy of GEBVs and GEGVs. Specifically, 1102 inbred lines were split into a training set comprising 80% (882 lines) and a testing set comprising 20% (220 lines). The phenotypic data was similarly divided into corresponding training and testing sets based on the inbred lines used across the four seasons. The GBLUP model was trained on the training set to predict the GEBVs and GEGVs. A k-fold cross-validation procedure (with k=5) was implemented to further partition the training set into k subsets. In each fold, the model was trained on k-1 subsets and used to predict GEBVs and GEGVs for the lines in the remaining subset. The prediction accuracy was evaluated using the correlation coefficient (r), which was calculated for each fold and averaged across the five folds, yielding a reliable estimate of the model's predictive capability. Cross-validation was conducted for six traits (AD, SD, TL, HC, EHP, and GY) due to insufficient data for other traits.

3.2.11 Calculation of environmental effects

The G x E BLUP model was used to calculate environmental effects and genotype by-environment interactions (GEI) effects, using the SNP Bayes factors to estimate the GEI effects and identify favourable environments. The model was used to calculate the testing environment on genotype expression with the following model:

$$y = Xb + Zu_{GxE} + Zu + e$$

Where: y is the vector of the observed phenotypic values; Xb = fixed effects (e.g., overall mean, environment effects); Z_{uGxE} = genotype x environment interaction effects (captures the overlap between environments); Zu = genetic effects (captures the genetic variation within environments), e is the vector of random errors with normal distribution of $N(0, I\,\sigma^2 e)$, where $\sigma^2 e$ is the residual variance and I is the identity matrix. The overlap between environments was captured through the genotype x environment interaction term (Zu_{GxE}) in the model equation.

3.2.12 Calculation of ecovalence

The ecovalence (W_i) for the hybrids were calculated using the following formula:

$$W_i = \Sigma_j (Y_{ij} - \bar{Y}_{i.} - \bar{Y}_{.j} + \bar{Y}_{..})^2$$

where, Y_{ij} is the predicted genotypic value of the i^th hybrid in the j^th year and $\bar{Y}_i.$ and $\bar{Y}_{.j}$ are the genotype and environment mean deviations respectively, and $\bar{Y}_{..}$ is the overall mean. For this reason, genotypes with a low W_i value have smaller deviations from the overall mean across environments and are thus more stable.

3.2.13 Genomic Data Analysis

The SNP data was analyzed using the COBALT software according to the Limagrain protocol (www.limagrain.com) to estimate the GEBVs and GEGVs.

3.3 Results

3.3.1 Genomic estimated breeding and genetic values for inbred lines.

GBLUP analysis was carried out on the genotypic data of groups 1 (N3 heterotic group) and 2 (SC heterotic group) inbred lines with computed GEBVs and GEGVs presented in Tables 3.4, 3.5, 3.6 and 3.7.

3.3.1.1 GEBVs for group 1 inbred lines

The best-selected 20 maize inbred lines and their GEBVs for agronomic traits and disease rating are presented in Table 3.4. Outstanding lines with the highest GEBVs (> 4.99 t/ha) for grain yield components and disease resistance were selected (Table 3.4). The line designated as G15NL310 had the lowest value for days to mid-pollen (66.40 days), followed by G15NL304 (66.70 days), TTN01 (68.83 days), G18NL248 (69.01 days) and 15AG143 (69.07 days) making them the top-performing early flowering lines. G15NL304 had the lowest days to mid-silking (67.82 days), followed by lines G15NL310 (67.95 days), GNL248 (69.65 days), 15AG143 (69.65 days) and TTN01 (69.79 days) in a desirable direction. The following lines had reduced anthesis to silking interval: CTL03 (0.18 days), 15AG162 (0.37 days), 15AG163 (0.45 days), GS-PL07 (0.47 days), 15AG111 (0.68 days) and GNL200 (0.76 days). Plant height ranged from 270.02 to 286.72 cm. The best lines with short plant height were G15NL310 (270.02cm), G18NL248 (274.46 cm), G16NL100 (276.18 cm), G17NL209 (276.97 cm) and GN18L200 (281.98 cm). Line G18NL248 had relatively the lowest ear height (133.57 cm), followed by G15NL310 (134.49 cm), G15NL304 (137.00 cm), G16NL100 (139.12 cm) and 15AG143 (139.39 cm) which are desirable selections. CTL03 had low and ideal stem lodging tolerance (4.71%), followed by lines G17NL200 (5.01%), G17NL201 (5.14%) and G17NL210 (5.46%). Further, G18NL200 and G17NL209 had the best total lodging tolerance value of 7.40%, followed by G17NL211 (7.50%), G17NL201 and G17NL210 both with 7.54%. For husk cover, G16NL99 with 1.99%, followed by CTL22 (2.20%), G16NL100 (2.94%) and 15AG163 (3.09%) were selected with low and ideal values. The highest number of plants per 6m^2 was computed for GS-PL07 at 25.71, followed by CTL03 (25.67), 15AG166 (25.57), CTL22 (25.54) and G15NL304 (25.52). Line CTL03 (23.74) had the highest number of ears harvested per 6m^2. Other top selections with higher ear numbers included GS-PL07 (23.19), CTL22 (22.64), G16NL99 (22.47) and G16NL100 (22.29). The mean number of ears per plant was 1.00 for the best lines. The GEBVs for grain yield varied from 4.99 t/ha to 5.55 t/ha. The highest GEBV for grain yield was computed for lines CTL03 at 5.55 t/ha, followed by GS-PL07 (5.28 t/ha), 15AG162 (5.23 t/ha), G15NL304 (5.10 t/ha) and 15AG111 (5.07 t/ha). The top 20 selected lines had a low maize streak virus score (1.00), while their *Phaesopharia* leaf spot reaction score was low and ranged between 1.80 and 3.21. *Phaesopharia*-resistant selection included CTL03 (with a disease score of 1.80), followed by 15AG162 (2.09), 15AG163 (2.20), G18NL248 (2.26) and 15AG1166 (2.33). Low values for GLS reaction were recorded for lines 15AG163 (1.61), 15AG162 (1.64), 15AG111 (1.76), CTL22 (1.76) and 15AG106 (1.77), priming them as the ideal selections. The mean leaf rust score of the best lines was relatively low at 1.65. The majority of the assessed traits exhibited moderate heritability (30-60%), suggesting better response to selection. Only SD, ASI and EPP recorded low heritability estimates, suggesting that these traits may not respond readily to direct selection. The means of the top 20 selected lines were higher than the grand means for most of the traits, except for ASI, SL, TL and PLS. Coefficient of determination (R^2) values were high (>60%) for most of the traits, except for ASI (51%) and MSV (28%).

Table 3.4 Genomic estimated breeding values for the best-selected 20 maize inbred lines from heterotic group 1 based on agronomic traits and major diseases rating.

	Agronomic traits												Disease parameters			
Lines	**AD**	**SD**	**ASI**	**PH**	**EH**	**SL**	**TL**	**HC**	**NP**	**EHP**	**EPP**	**GY**	**MSV**	**PLS**	**GLS**	**RST**
CTL03	70.14	70.54	0.18	286.72	148.36	4.71	9.36	4.73	25.67	23.74	1.02	5.55	1.08	1.80	1.86	1.65
GS-PL07	69.85	70.33	0.47	281.82	144.55	6.07	9.60	4.95	25.71	23.19	1.00	5.28	1.04	2.38	1.81	1.65
15AG162	69.90	70.25	0.37	282.39	141.37	6.02	9.49	3.20	25.32	21.71	0.98	5.23	1.03	2.09	1.64	1.65
15AG163	69.78	70.17	0.45	282.16	139.66	6.29	9.92	3.09	25.08	21.72	0.98	5.15	1.02	2.20	1.61	1.64
G15NL304	66.70	67.82	1.13	278.50	137.00	7.38	9.40	13.27	25.52	22.26	0.97	5.10	0.98	3.18	1.79	1.64
15AG111	69.64	70.24	0.68	281.98	143.55	7.44	11.22	13.02	25.18	22.14	0.99	5.07	1.03	2.85	1.76	1.65
15AG143	69.07	69.65	1.05	277.68	139.39	6.39	8.45	3.63	25.21	21.54	0.97	5.06	0.99	2.83	1.96	1.64
CTL22	69.53	70.37	0.88	280.25	142.33	6.72	9.15	2.20	25.54	22.64	0.98	5.04	1.01	2.55	1.76	1.65
G16NL100	69.77	70.62	0.90	276.18	139.12	6.39	8.05	2.94	25.33	22.29	0.96	5.03	0.99	2.55	1.85	1.64
G16NL99	69.65	70.51	0.92	277.34	139.71	6.36	8.02	1.99	25.43	22.47	0.97	5.03	0.98	2.55	1.85	1.64
15AG105	69.37	70.07	0.77	282.54	143.32	6.71	10.23	8.57	25.32	22.04	0.98	5.02	1.02	2.65	1.78	1.65
TTN01	68.83	69.79	0.89	281.64	141.20	6.73	8.51	24.88	25.15	21.56	0.97	5.01	0.99	2.74	1.89	1.64
G15NL310	66.40	67.95	1.21	270.02	134.49	7.53	9.45	11.72	25.50	21.70	0.96	5.01	0.98	3.21	1.79	1.64
G17NL209	69.92	70.19	0.82	276.97	139.60	5.59	7.40	4.79	25.29	21.08	0.96	5.00	0.99	2.42	1.88	1.64
G18NL200	70.05	70.10	0.76	277.10	140.71	5.01	7.40	10.05	25.29	22.02	0.96	5.00	0.99	2.53	1.89	1.64
G18NL248	69.01	69.65	0.98	274.46	133.57	5.58	8.37	4.96	24.70	21.41	0.97	5.00	0.98	2.26	1.78	1.64
G17NL211	70.42	70.55	0.83	279.49	140.60	5.53	7.50	5.15	25.36	21.45	0.97	5.00	1.00	2.41	1.88	1.64
G17NL210	70.41	70.53	0.83	279.77	140.67	5.46	7.54	6.25	25.26	21.51	0.97	5.00	1.00	2.43	1.88	1.64
15AG166	69.56	70.40	0.78	280.01	142.55	6.01	9.43	4.97	25.57	21.48	0.98	5.00	1.03	2.33	1.77	1.65
G17NL201	69.98	70.12	0.78	277.68	140.53	5.14	7.54	9.93	25.21	22.01	0.97	4.99	0.99	2.52	1.89	1.64
Heritability	0.32	0.21	0.10	0.33	0.30	0.40	0.34	0.54	0.56	0.32	0.19	0.51	0.52	0.39	0.42	0.54
Mean of selected lines	69.40	69.99	0.78	279.24	140.61	6.15	8.80	7.21	25.33	22.00	0.98	5.08	1.01	2.52	1.82	1.64
Grand mean	68.67	69.72	1.12	276.80	138.00	6.61	8.81	7.36	25.08	20.75	0.96	4.76	0.98	2.63	1.78	1.64
Std Dev	4.98	4.73	1.28	62.99	32.07	9.05	11.53	9.06	7.06	8.96	0.38	3.09	0.23	1.47	0.96	0.82
Std Error	0.62	0.79	0.29	6.75	4.28	2.23	2.60	6.82	0.81	1.94	0.04	0.24	0.04	0.50	0.17	0.04
Minimum	66.16	67.61	0.18	265.24	130.84	4.71	5.85	-1.00	23.29	15.08	0.90	4.14	0.97	1.80	1.55	1.63
Maximum	70.64	71.16	1.66	287.37	148.36	12.95	16.97	34.58	26.72	24.28	1.02	5.55	1.08	4.66	1.96	1.65
R^2	0.84	0.79	0.51	0.96	0.93	0.73	0.70	0.62	0.79	0.81	0.84	0.89	0.28	0.82	0.75	0.78

AD=Days to mid-pollen; SD=Days to mid-silking; ASI=Anthesis to silking interval (days); PH= Plant height (centimetres); EH=Ear height (centimetres); SL=stem lodging (percentage); TL=Total lodging (percentage); HC=Husk cover (percentage); NP=Number of plants per $6m^2$; EHP=number of ears harvested per $6m^2$; EPP= number of ears per plant; GY=Grain yield (t/ha); MSV=Maize streak virus; PLS=*Phaesopharia* leaf spot; GLS=Grey leaf spot; RST=Rust; Std Dev =Standard deviation, Std Error= Standard Error, R^2=Coefficient of determination

3.3.1.2 GEGVs for group 1 inbred lines

The summary of the genomic estimated genetic values for the best-selected 20 inbred lines from heterotic group 1 based on agronomic traits and major disease ratings are presented in Table 3.5. The lowest days to mid-pollen were recorded for G15NL346 with 69.97 days, followed by G15NL310 (67.07 days), G15NL304 (67.46 days), TTN01 (69.08 days) and G18NL248 (69.32 days). Lines with the lowest days to mid-silking included CTL03 (68.86 days), 15AG143 (68.89 days), TTN01 (69.80 days), G18NL248 (69.86 days) and G17NL209 (70.30 days). The best selections had mid-silking values ranging from 68.86 to 71.37 days. The lowest ASI were recorded for CTL03 (0.18 days), 15AG162 (0.37 days), 15AG163

(0.45 days), GS-PL07 (0.47 days) and G17NL200 (0.76 days) among the top selections. The ASI values were relatively low and ideal for all the best-selected lines, ranging from 0.18 to 1.50. Line G18NL248 had the shortest plant height at 101.02 cm. Other lines with short plant height included G15NL346 (101.29 cm), G15NL310 (102.87 cm), G15NL304 (106.16 cm) and G16NL100 (106.52 cm). Low and desirable values for stem lodging tolerance were recorded for CTL03 (with lodging of 4.78%), G17NL200 (6.95%), G17NL211 (6.98%), G17NL210 (7.01%) and G17NL201 (7.03%). Lines G16NL99 (with a husk cover of 1.99%), CTL22 (2.20%), G16NL100 (2.94%) and 15AG163 (3.09%) had the lowest values in a desirable direction. Husk cover ranged from 1.99 (G16NL99) to 24.88% (TTN01). The number of ears harvested per 6m^2 varied from 23.69 (G18NL248) to 26.05 (CTL03). Lines CTL03 (with 26.05 ears per 6m^2), followed by GS-PL07 (25.93), G15NL346 (25.56) and G16NL99 (25.15) were the best selections. The mean GEGVs for the number of ears per plant was 1.00 for the top selections. The best selections had relatively high GEGVs (> 5.86 t/ha) for grain yield. The highest GEGV for grain yield was computed for CTL03 with 6.12 t/ha followed by GS-PL07 (5.99 t/ha), 15AG162 (5.94t/ha) and both G15NL304 and 15AG163 recording 5.90t/ha. GEGVs for grain yield ranged between 5.86 (G17NL213) and 6.12 t/ha (CTL03). The mean MSV score was 1.00 for the top selections. Low *Phaesopharia* leaf spot scores were noted for G18NL248 (with a score of 1.80), G15NL310 (2.09), TTN01 (2.20), G17NL209 (2.26) and G15NL304 (2.38). The grey leaf spot ratings were < 2.00 for the top lines. GLS scores ranged between 1.62 (15AG163) and 1.96 (15AG143). Variable heritability estimates were recorded for the assessed traits varying from 10% (ASI) to 54% (HC). The traits mean of the top selected lines had higher values than the grand means. The coefficient of determination for the assessed traits varied from 4% (MSV) to 38% (SL).

Table 3.5 The best-selected 20 maize inbred lines from heterotic group 1 based on genomic estimated genetic values for agronomic traits and major diseases rating.

	Agronomic traits										Disease parameters		
Lines	**AD**	**SD**	**ASI**	**EH**	**SL**	**TL**	**HC**	**EHP**	**EPP**	**GY**	**MSV**	**PLS**	**GLS**
CTL03	70.40	68.86	0.18	116.40	4.78	8.69	4.73	26.05	1.02	6.12	1.08	1.80	1.85
GS-PL07	70.22	70.39	0.47	114.82	6.07	8.68	4.95	25.93	1.00	5.99	1.04	2.38	1.82
15AG162	70.10	71.37	0.37	109.23	5.99	8.56	3.20	24.15	0.98	5.94	1.03	2.09	1.64
15AG163	69.69	70.40	0.45	107.67	6.25	8.80	3.09	24.40	0.98	5.90	1.02	2.20	1.62
G15NL304	67.46	71.23	1.13	106.16	7.20	8.26	13.27	24.12	0.97	5.90	0.98	3.18	1.79
15AG143	69.38	68.89	1.05	107.46	6.31	7.72	3.63	24.22	0.97	5.88	0.99	2.83	1.96
G16NL100	70.06	71.09	0.90	106.52	6.31	7.38	2.94	25.02	0.96	5.88	0.99	2.55	1.85
TTN01	69.08	69.80	0.89	108.36	6.66	7.74	24.88	23.85	0.97	5.88	0.99	2.74	1.88
G16NL99	69.95	70.84	0.92	107.25	6.27	7.35	1.99	25.15	0.97	5.88	0.98	2.55	1.85
G15NL310	67.07	71.18	1.21	102.87	7.34	8.26	11.72	23.72	0.96	5.88	0.98	3.21	1.80
CTL22	69.93	71.16	0.88	111.78	6.67	8.31	2.20	24.79	0.98	5.87	1.01	2.55	1.77
G15NL346	66.97	70.59	1.50	101.29	5.92	7.66	6.89	25.56	0.96	5.87	0.98	3.11	1.74
G17NL209	70.09	70.30	0.82	106.56	5.52	6.93	4.79	23.94	0.96	5.87	0.99	2.42	1.88
G17NL211	70.54	70.64	0.83	107.90	5.46	6.98	5.15	24.26	0.97	5.87	1.00	2.41	1.88
G17NL210	70.54	70.87	0.83	107.95	5.39	7.01	6.25	24.32	0.97	5.87	1.00	2.43	1.88
G17NL200	70.10	70.70	0.76	107.27	4.96	6.95	10.05	24.92	0.96	5.86	0.99	2.53	1.89
G18NL248	69.32	69.86	0.98	101.02	5.48	7.59	4.96	23.69	0.97	5.86	0.98	2.26	1.78
G17NL201	70.10	70.78	0.78	107.24	5.08	7.03	9.93	24.79	0.97	5.86	0.99	2.52	1.89
G17NL212	69.81	70.94	0.90	108.10	5.41	7.08	5.56	24.48	0.97	5.86	0.99	2.50	1.90
G17NL213	69.59	70.46	0.88	107.85	5.44	7.08	5.69	24.67	0.97	5.86	0.99	2.47	1.88
Heritability	0.32	0.21	0.10	0.30	0.40	0.34	0.54	0.32	0.19	0.51	0.52	0.39	0.42
Mean of selected lines	69.52	70.52	0.84	107.69	5.93	7.70	6.79	24.60	0.97	5.89	1.00	2.54	1.83
Grand Mean	69.14	70.56	1.12	106.61	6.48	7.89	7.36	23.09	0.96	5.75	0.98	2.63	1.78
Std Deviation	5.17	5.12	1.28	51.59	9.02	11.38	9.06	9.10	0.38	3.11	0.23	1.47	0.96
Std Error	0.81	0.76	0.29	4.53	2.20	2.04	6.82	1.91	0.04	0.18	0.04	0.50	0.17
Minimum	66.77	68.55	0.18	98.53	4.78	5.91	0.08	17.59	0.90	5.46	0.97	1.80	1.56
Maximum	70.98	71.92	1.66	117.68	12.86	13.94	34.58	26.40	1.02	6.12	1.08	4.66	1.96
R^2	0.37	0.32	0.19	0.23	0.38	0.09	0.34	0.30	0.08	0.13	0.04	0.23	0.08

AD=Days to mid-pollen; SD=Days to mid-silking; ASI=Anthesis to silking interval (days); EH=Ear height (centimetres); SL=Stem lodging (percentage); TL=Total lodging (percentage); HC=Husk cover (percentage); EHP=number of ears harvested per $6m^2$; EPP= number of ears per plant; GY=Grain yield (t/ha); MSV=Maize streak virus; PLS=*Phaesopharia* leaf spot; GLS=Grey leaf spot; Std Deviation= Standard deviation; ; Std Error= Standard error; R^2=Coefficient of determination

3.3.1.3 GEBVs for group 2 inbred lines

Table 3.6 presents the best-selected 20 maize lines based on GEBVs for grain yield and major diseases. Mid-pollen shedding varied from 67.46 (G16NL724) to 70.19 (G16NL860) days. The lowest days to mid-pollen shedding were recorded for G16NL72 (67.46 days), G17NL642 (67.69 days), GS-Pl26 (67.83 days), G16NL721 (67.95 days) and G16NL898 (67.97 days). Similarly, the shortest days to mid-silking were noted for G16NL898 (68.83 days), GNL642 (68.94 days), G16NL724 (69.20 days), GS-PLS26 (69.20 days) and G16NL777 (69.50 days) for the top selections. Lines with short plant height were GL16NL805 at 271.17 cm, followed by G16NL898 (271.55 cm), G16NL806 (271.66 cm), G16NL747 (276.98 cm) and G16NL777 (277.11 cm). Lines with reduced ear height GEGVs included G16NL898

(132.83 cm), G16NL805 (135.91cm), GS-PLS29 (136.44 cm), G16NL817 (136.53 cm) and G16NL806 (136.71cm). Stem lodging tolerant lines were G16NL724 (with lodging of 1.58%), G16NL721 (2.00%), G16NL777 (2.51%), G16NL834 (3.89%) and G16NL805 (4.23%). Lines G16NL819 (1.12%), G16NL898 (1.63%), G16NL684 (1.76%), G16NL806 (1.91%) and G16NL805 (2.16%) were relatively tolerant to root lodging. Top selections with total lodging tolerance included G16NL724 (with lodging of 3.16%), G16NL777 (3.44%), G16NL898 (3.64%), G16NL721 (3.96%) and G16NL806 (4.12%). Low husk cover GEGVs were recorded for GS-PL26 (5.52%), G16NL684 (5.92%), G16NL819 (6.06%), G16NL679 (6.34%) and G16NL817 (6.36%) in a desirable direction. The mean number of plants per 6m^2 was 25.00 for the top selected lines. G16NL777 (with 23.53 plants per 6m^2), was the top selection, followed by G16NL684 (22.78), G16NL679 (22.24), G16NL805 (22.2) and G16NL89 (22.18). The mean number of ears per plant was 1.00 for the top 20 selections. The GEBVs for grain yield varied from 5.52 t/ha (G16NL724) to 5.96 t/ha (G17NL544). The highest GEBVs for grain yield were noted for two lines (G17NL544 and GS-PL26) with 5.96 t/ha. Other top-performing lines with high GEGVs for grain yield included G16NL721 (5.82 t/ha), G16NL819 (5.8 t/ha), G17NL642 (5.79 t/ha) and G16NL860 5.77 (t/ha). GEBVs for maize streak virus were low for the top 20 selections, ranging from 0.93 (G17NL642) and 1.08 (GS-PL29). The following lines were tolerant to *Phaesopharia* leaf spot: G16NL816 (with a rating of 2.25), G16NL747 (2.34), G16NL827 (2.37) and G16NL834 (2.37) in descending order. The mean leaf rust score was < 2.00 for the top selected lines. Lines G16NL805 (with a score of 1.03), G16NL806 (1.18), G16NL684 (1.37), G16NL679 (1.39) and G17NL642 (1.41) were tolerant to GLS. The assessed traits exhibited moderate heritability (>30%) except for SD, HC and EPP, which recorded low heritability values. The means of selected lines were higher than the grand means for most of the evaluated traits, except for SL, RL, TL, HC, PLS and RST. High coefficient of determination (>60%) were computed for most of the traits except RL (45%) and MSV (28%).

Table 3.6 Genomic estimated breeding values for the best-selected 20 maize inbred lines from heterotic group 2 based on agronomic traits and major diseases rating.

	Agronomic traits												Disease parameters			
Lines	**AD**	**SD**	**PH**	**EH**	**SL**	**RL**	**TL**	**HC**	**NP**	**EHP**	**EPP**	**GY**	**MSV**	**PLS**	**RST**	**GLS**
G17NL544	68.22	69.65	280.30	141.10	8.08	4.22	10.04	6.53	25.32	21.23	0.98	5.96	0.98	2.73	1.63	2.10
GS-PL26	67.83	69.20	285.13	139.07	6.08	3.04	10.72	5.52	25.25	22.15	0.99	5.96	1.03	3.22	1.69	1.92
G16NL721	67.95	69.54	279.48	138.54	2.00	3.18	3.96	7.05	25.32	20.49	0.95	5.82	0.98	2.63	1.73	2.50
G16NL819	68.93	70.01	282.82	137.09	4.53	1.12	5.27	6.06	25.40	21.97	1.05	5.80	0.96	2.61	1.38	2.41
G17NL642	67.69	68.94	282.20	139.18	7.63	3.16	10.16	6.97	25.44	22.16	0.95	5.79	0.93	2.58	1.62	1.41
G16NL860	70.19	70.90	283.90	142.15	6.07	3.29	10.56	6.63	25.60	21.91	0.99	5.77	0.98	2.58	1.58	1.80
GS-PL29	68.41	69.72	281.23	136.44	8.89	2.76	9.47	14.54	25.53	21.83	0.92	5.77	1.08	2.70	1.98	1.72
G16NL816	69.22	69.91	281.79	140.13	4.26	3.27	6.68	6.87	25.09	20.95	0.99	5.76	0.97	2.25	1.50	2.89
G16NL684	69.83	70.14	283.73	142.56	4.80	1.76	7.27	5.92	25.79	22.78	0.95	5.73	0.97	2.41	1.89	1.37
G16NL679	69.33	69.94	283.05	143.06	6.08	3.01	9.97	6.34	25.50	22.24	0.99	5.65	1.00	2.38	1.62	1.39
G16NL817	68.60	69.76	279.03	136.52	5.23	2.99	8.04	6.36	24.75	20.89	1.06	5.62	0.95	2.39	1.41	2.08
G16NL777	68.57	69.50	277.11	137.88	2.51	3.38	3.44	7.90	25.24	23.53	1.05	5.59	0.99	2.64	1.69	2.77
G16NL805	68.29	69.53	271.17	135.91	4.23	2.16	4.42	6.93	25.41	22.20	0.98	5.58	0.98	3.10	1.49	1.03
G16NL827	69.47	69.90	278.22	139.12	10.69	3.55	13.36	7.46	24.94	21.47	1.04	5.58	1.00	2.37	1.46	2.33
G16NL806	68.47	69.72	271.66	136.71	4.33	1.91	4.12	6.65	25.35	22.06	0.98	5.56	0.98	3.05	1.52	1.18
G16NL898	67.97	68.83	271.55	132.83	4.42	1.63	3.64	8.70	25.21	22.18	0.92	5.55	0.98	2.82	1.67	1.70
G16NL915	70.01	70.12	278.74	138.53	4.48	3.12	6.12	6.45	25.19	21.47	1.03	5.54	0.96	2.46	1.51	1.96
G16NL834	70.06	70.09	279.83	138.52	3.89	3.54	6.75	6.52	25.09	21.60	1.04	5.54	0.96	2.37	1.55	2.00
G16NL747	69.39	70.21	276.98	137.14	10.00	3.32	11.83	7.19	25.08	21.40	1.01	5.52	0.97	2.34	1.58	2.56
G16NL724	67.46	69.20	278.29	138.54	1.58	2.94	3.16	6.79	25.32	20.38	0.95	5.52	0.97	2.66	1.92	2.03
Heritability	0.32	0.21	0.33	0.30	0.54	0.40	0.34	0.22	0.56	0.32	0.19	0.51	0.54	0.52	0.39	0.42
Mean of selected lines	68.79	69.74	279.30	138.60	5.49	2.87	7.45	7.17	25.29	21.74	0.99	5.68	0.98	2.61	1.62	1.96
Grand Mean	68.67	69.72	276.80	138.00	6.61	3.50	8.81	7.36	25.08	20.74	0.96	4.76	0.98	2.63	1.64	1.78
Std Deviation	4.98	4.73	62.99	32.07	0.82	9.05	11.53	1.28	7.06	8.96	0.38	3.09	9.06	0.23	1.47	0.96
Std Error	0.46	0.38	4.36	3.08	2.98	1.59	3.66	1.53	0.34	1.12	0.05	0.35	0.05	0.26	0.16	0.35
Minimum	67.37	68.79	260.20	126.50	0.00	0.66	1.52	5.52	24.08	18.02	0.82	2.38	0.93	1.79	1.37	0.77
Maximum	71.04	71.27	289.20	148.20	22.22	9.83	29.80	14.50	25.91	23.53	1.09	5.96	1.08	3.31	2.01	3.67
R^2	0.84	0.79	0.96	0.93	0.73	0.45	0.70	0.62	0.79	0.81	0.84	0.89	0.28	0.82	0.78	0.75

AD=Days to mid-pollen; SD=Days to mid-silking; PH= Plant height (centimetres); EH=Ear height (centimetres); SL=Stem lodging (percentage); RL=Root lodging (percentage); TL=Total lodging (percentage); HC=Husk cover (percentage); NP = Number of plants per $6m^2$; EHP = number of ears harvested per $6m^2$; EPP= number of ears per plant; GY=Grain yield (t/ha); MSV=Maize streak virus; PLS=*Phaesopharia* leaf spot; GLS=Grey leaf spot; RST=Rust; Std Deviation= Standard deviation, Std Error= Standard error; R^2=Coefficient of determination

3.3.1.4 GEGVs for group 2 inbred lines

The GEGVs for the top-performing 20 maize lines for agronomic traits and major disease ratings are presented in Table 3.7. Top-performing lines with the lowest days to mid pollen included G16NL724 (with a value of 67.90 days), followed by GS-PL26 (68.01 days), G17NL642 (68.18 days), G16NL721 (68.35 days) and G17NL544 (68.51 days) confirming them as the most desirable early flowering lines. Line G17NL564 is the best selection with a reduced time for mid-silking (69.44 days), followed by G17NL642 (69.64 days), GS-PL26 (69.71 days), G16NL724 (69.98 days) and G17NL544 (70.29 days). Good stem lodging tolerance was noted in lines G16NL724 with 1.49%, followed by G16NL721 (1.94%), G16NL777

(2.32%), G16NL834 (3.72%) and G16NL804 (3.83%). Stem lodging tolerance scores varied from 1.94% (G16NL721) to 10.58% (G16NL827). The following top lines displayed good root lodging tolerance: GS-PL29 (3.42%), G16NL819 (3.72%), G16NL915 (4.21%), G16NL777 (4.24%) and G16NL721 (4.26%). Similarly, lines G16NL724 with 2.75%, followed by G16NL777 (3.00%), G16NL721 (3.48%), G16NL806 (4. 16%) were tolerant to total lodging. The best lines with good husk cover were G16NL804 (4.22%), GS-PL26 (5.52%), G16NL804 (5.92%), G16NL819 (6.06%), G16NL679 (6.34%) and G16NL817 (6.36%) among the top-selected lines. Mean husk cover ranged from 5.52% (GS-PL26) to 14.54% (GS-PL29). Line GS-PL29, with a value of 29.40, exhibited the highest number of plants per 6m^2. The other top selections included G16NL684 (28.99), G16NL819 (28.97), GNL544 (28.95) and G16NL860 (28.93) in descending order. G16NL777 had the highest number of ears per 6m^2 (25.90), followed by G17NL564 (25.71), G16NL684 (25.03), G16NL805 (24.68) and G17NL642 (24.64), making them the top selections for the trait. The mean number of ears per plant was 1.00 for all the top 20 lines. Grain yield was high for the top selections (>6.46 t/ha). The highest GEGVs for grain yield were computed for G17NL544 with 6.81 t/ha, followed by G16NL721 (6.74 t/ha), G17NL642 (6.72 t/ha), G16NL860 (6.66 t/ha), G16NL679 (6.65 t/ha) and GS-PL26 (6.63 t/ha) making them best candidates for hybrid breeding. The top-selected lines exhibited good tolerance to maize streak virus (a mean of 1.00). Similarly, *Phaesopharia* leaf spot values were < 3.00 for the top-selections. The lines showing the highest tolerance to *Phaesopharia* leaf spot included G16NL816 (2.25), G16NL827 (2.37), G16NL834 (2.37), G16NL679 (2.38) and G16NL817 (2.39) in descending order. Leaf rust scores varied between 1.49 (G16NL819) to 2.52 (GS-PL26), while GLS rating ranged between 1.06 (G16NL804) to 2.92 (G16NL816). Heritability estimates were moderately high (30-60%) for most of the traits. The coefficient of determination value was relatively lower (<47%) for most of the assessed traits, except for AD and SL (each with 52%).

Table 3.7 Genomic estimated genetic values for the best-selected 20 maize inbred lines from heterotic group 2 based on agronomic traits and foliar disease rating.

	Agronomic traits										**Disease parameters**			
Lines	**AD**	**SD**	**SL**	**RL**	**TL**	**HC**	**NP**	**EHP**	**EPP**	**GY**	**MSV**	**PLS**	**RST**	**GLS**
G17NL544	68.51	70.29	7.97	4.98	9.77	6.53	28.95	23.71	0.98	6.81	0.98	2.73	1.72	2.07
G16NL721	68.35	70.30	1.94	4.26	3.48	7.05	28.73	22.62	0.95	6.74	0.98	2.63	1.86	2.51
G17NL642	68.18	69.64	7.61	4.53	8.85	6.97	28.81	24.64	0.95	6.72	0.93	2.58	1.74	1.40
G16NL860	70.66	71.89	5.79	4.36	9.37	6.63	28.93	23.88	0.99	6.66	0.98	2.58	1.71	1.86
G16NL679	69.77	70.75	5.84	4.66	8.65	6.34	28.48	24.55	0.99	6.65	1.00	2.38	1.74	1.43
GS-PL26	68.01	69.71	6.88	4.40	10.60	5.52	28.49	24.61	0.99	6.63	1.03	3.22	2.52	1.88
G16NL819	69.30	70.89	4.36	3.72	4.77	6.06	28.97	24.24	1.05	6.63	0.96	2.61	1.49	2.43
G16NL816	69.62	70.80	4.12	4.68	6.34	6.87	28.79	23.01	0.99	6.62	0.97	2.25	1.61	2.92
G16NL684	70.25	71.00	4.61	4.29	6.28	5.92	28.99	25.03	0.95	6.60	0.97	2.41	2.00	1.41
G17NL564	68.63	69.44	6.17	4.26	6.50	6.69	28.86	25.71	0.97	6.60	0.97	2.64	1.68	1.55
G16NL777	69.02	70.33	2.32	4.24	3.09	7.90	28.82	25.90	1.05	6.54	0.99	2.64	1.80	2.76
G16NL805	68.73	70.42	4.01	4.26	4.53	6.93	28.85	24.68	0.98	6.54	0.98	3.10	1.57	1.05
G16NL817	68.93	70.65	5.09	4.61	7.39	6.36	28.65	23.03	1.06	6.52	0.95	2.39	1.54	2.10
G16NL806	68.87	70.61	4.14	4.36	4.16	6.65	28.81	24.55	0.98	6.51	0.98	3.05	1.59	1.19
G16NL827	69.88	70.76	10.58	4.62	12.14	7.46	28.81	23.67	1.04	6.49	1.00	2.37	1.59	2.34
G16NL834	70.55	71.04	3.72	4.68	6.00	6.52	28.71	23.83	1.04	6.48	0.96	2.37	1.65	2.02
G16NL915	70.50	71.09	4.31	4.21	5.44	6.45	28.89	23.60	1.03	6.48	0.96	2.46	1.62	1.98
G16NL724	67.90	69.98	1.49	4.26	2.75	6.79	28.73	22.47	0.95	6.47	0.97	2.66	2.04	2.05
GS-PL29	68.99	70.64	8.70	3.42	8.58	14.54	29.40	23.66	0.92	6.47	1.08	2.70	2.13	1.75
G16NL804	69.11	70.46	3.83	4.48	4.22	6.73	28.85	24.48	0.97	6.46	0.98	3.04	1.60	1.06
Heritability	0.32	0.21	0.54	0.40	0.34	0.10	0.56	0.54	0.19	0.51	0.54	0.52	0.39	0.42
Mean of selected lines	69.19	70.53	5.17	4.36	6.65	7.05	28.83	22.97	0.99	6.58	0.98	2.64	1.76	1.89
Grand Mean	69.14	70.56	6.48	4.41	7.89	7.36	28.73	23.09	0.96	5.75	0.98	2.63	1.74	1.78
Std Dev	5.17	5.12	9.02	6.61	11.38	9.06	7.13	9.10	0.38	3.11	0.23	1.47	0.89	0.96
Std Error	0.60	0.55	2.66	0.86	2.90	3.71	0.52	1.46	0.05	0.28	0.05	0.36	0.12	0.27
Minimum	67.85	69.44	0.85	3.42	1.20	5.52	27.80	20.08	0.82	3.48	0.93	1.79	1.47	0.80
Maximum	71.56	73.32	22.06	5.68	27.67	14.54	29.40	25.90	1.09	6.81	1.08	3.31	2.52	3.67
R^2	0.52	0.47	0.52	0.03	0.31	0.25	0.10	0.30	0.18	0.42	0.10	0.25	0.14	0.35

AD=Days to mid-pollen; SD=Days to mid silking; SL=Stem lodging (percentage); RL=Root lodging (percentage); TL=Total lodging (percentage); HC=Husk cover (percentage); NP=Number of plants per $6m^2$; EHP=number of ears harvested per $6m^2$; EPP= number of ears per plant; GY=Grain yield (t/ha); MSV=Maize streak virus; PLS=*Phaesopharia* leaf spot; RST=Rust; GLS=Grey leaf spot; Std Dev=Standard deviation; Std Error = Standard error; R^2=Coefficient of determination

3.3.1.5 GEGVs for top-performing hybrids

Table 3.8 presents the best 20 selected maize hybrids consisting of lines from both the N3 and SC heterotic groups for agronomic traits and foliar disease rating based on genomic estimated genetic values. The days to mid-pollen shedding in the selected hybrids varied from 69.16 days (hybrid coded as G17NL642 x TTN01) to 71.65 days (G16NL860 x TTN01). Early flowering hybrids with relatively reduced days to mid pollen shedding were G17NL642 x TTN01 (69.16 days), SC659 (69.29 days), G16NL721 x TTN01 (69.42 days), G16NL898 x TTN01 (69.43 days) and G17NL544 x TTN01 (69.68 days), Low GEGVs in a desirable state for days to mid-silking were noted for hybrid G16NL898 x TTN01 (69.64 days), followed by G17NL642 x TTN01 (69.76 days), SC659 (70.02 days), G16NL777 x TTN01 (70.31 days) and G16NL805 x TTN01 (70.34 days). The best hybrids with short plant height included G16NL805 x TTN01 (281.09 cm), followed by G16NL898 x TTN01 (281.47 cm), G16NL806 x TTN01 (281.58 cm), G16NL747 x TTN01 (286.90 cm) and G16NL777 x TTN01 (287.02 cm) in a desired direction. The following hybrids recorded low ear height: G16NL898 x TTN01 (143.19 cm), G16NL805 x TTN01 (146.27 cm), SC727 (146.80 cm), G16NL817 x TTN01 (146.88 cm) and G16NL806 x TTN01 (147.07 cm). Single cross hybrids tolerant to stem lodging were G16NL721 x TTN01 (with stem lodging of 0.10%), G16NL777 x TTN01 (0.61%), 17C4292 (1.99%), G16NL805 x TTN01 (2.33%) and G16NL816 x TTN01 (2.37%). Similarly, low root lodging tolerance values were recorded for hybrids, namely G16NL819 x TTN01 (1.64%), G17NL237 x TTN01 (1.64%), SC727 (2.68%), G16NL898 x TTN01 (3.24%) and G16NL915 x TTN01 (3.26%). Total plant lodging varied from 4.00% (G16NL777 x TTN01) to 13.91% (G16NL827 x TTN01). The hybrid designated as G16NL777 x TTN01 was relatively tolerant to total lodging (4.00%), followed by G16NL898 x TTN01 (4.20%), G16NL721 x TTN01 (4.52%), G16NL806 x TTN01 (4.68%) and G16NL805 x TTN01 (4.97%). Hybrids SC659 (2.89%), G16NL684 x TTN01 (3.29%), G16NL819 x TTN01 (3.42%), G17NL237 x TTN01 (3.42%), and G16NL679 x TTN01 (3.71%) recorded the lowest husk cover values among the top-selected hybrids. The number of plants per 6m^2 varied between 26.58 (G16NL747 x TTN01) to 29.44 (SC727). Hybrids with the highest GEGVs for the number of plants per 6 m^2 included SC727 (29.44), G16NL684 x TTN01 (28.66), G16NL819 x TTN01 (28.65), G17NL544 x TTN01 (28.56), G16NL860 x TTN01 (28.53) and G16NL898 x TTN01 (28.51). The highest GEGVs for ears harvested per 6m^2 were computed for G16NL777 x TTN01 (26.53), G16NL684 x TTN01 (25.78), G16NL679 x TTN01 (25.24), G16NL805 x TTN01 (25.20) and G16NL898 x TTN01 (25.18) in a desirable direction. GEGVs for grain yield varied from 6.31 t/ha (G16NL747 x

TTN01) to 6.75 t/ha (G17NL544 x TTN01 and SC659). The highest-yielding hybrids were G17NL544 x TTN01 and SC659 (both with 6.75 t/ha), followed by G16NL721 x TTN01 (6.60 t/ha), then G16NL819 x TTN01 and G17NL237 x TTN01 (both with 6.59 t/ha). The top 20 hybrid selections exhibited excellent maize streak virus tolerance scoring from 1.03 (G17NL642 x TTN01) to 1.18 (SC727). Low *Phaesopharia* leaf spot GEGVs were recorded for G16NL816 x TTN01 (1.42), G16NL747 x TTN01 (1.51), G16NL834 x TTN01 and G16NL827 x TTN01 (each 1.54), and G16NL679 x TTN01 (1.55). The leaf rust rating was low (< 2.00) for all the top 20 selected hybrids, with values ranging between 1.39 (G16NL819 x TTN01) and 1.98 (SC727). The following hybrids were GLS tolerant: G16NL805 x TTN01 (1.11), G16NL806 x TTN01 (1.25), G16NL684 x TTN01 (1.45), G16NL679 x TTN01 (1.47) and G17NL642 x TTN01 (1.49). The broad-sense heritability values were moderate to high (32 to 67%), resolving better repeatability. The means of the selected lines were higher than the grand means for most of the assessed traits. High coefficient of determination values were recorded (> 60%), except for RL (41%) and MSV (32%). The values ranged from 32% (MSV) to 96% (PH).

Table 3.8 The top-performing 20 maize hybrids based on genomic estimated genetic values for agronomic traits and foliar disease rating.

	Agronomic traits											**Disease parameters**			
Crosses	**AD**	**SD**	**PH**	**EH**	**SL**	**RL**	**TL**	**HC**	**NP**	**EHP**	**GY**	**MSV**	**PLS**	**RST**	**GLS**
G17NL544 x TTN01	69.68	70.46	290.26	151.41	6.18	6.69	10.59	3.89	28.56	24.22	6.75	1.08	1.90	1.63	2.17
SC659	69.29	70.02	295.05	149.42	4.18	4.48	11.27	2.89	28.19	25.15	6.75	1.12	2.39	1.69	2.00
G16NL721 x TTN01	69.42	70.35	289.39	148.90	0.10	3.43	4.52	4.41	27.81	23.49	6.60	1.08	1.80	1.74	2.57
G16NL819 x TTN01	70.40	70.82	292.74	147.45	2.64	1.64	5.82	3.42	28.65	24.96	6.59	1.05	1.78	1.39	2.49
G17NL237 x TTN01	70.40	70.82	292.74	147.45	2.64	1.64	5.82	3.42	28.04	24.96	6.59	1.05	1.78	1.39	2.49
G17NL642 x TTN01	69.16	69.76	292.11	149.53	5.73	4.85	10.71	4.33	28.09	25.15	6.57	1.03	1.75	1.62	1.49
G16NL860 x TTN01	71.65	71.72	293.82	152.51	4.17	3.96	11.11	3.99	28.53	24.90	6.55	1.08	1.75	1.58	1.88
SC727	69.87	70.53	291.14	146.80	7.00	2.68	10.03	11.90	29.44	24.83	6.55	1.18	1.87	1.98	1.80
G16NL816 x TTN01	70.68	70.73	291.71	150.49	2.37	5.73	7.23	4.23	28.01	23.94	6.55	1.06	1.42	1.50	2.97
G16NL684 x TTN01	71.29	70.95	293.65	152.91	2.90	3.75	7.83	3.29	28.66	25.78	6.52	1.06	1.58	1.90	1.45
G16NL679 x TTN01	70.79	70.76	292.97	153.42	4.18	5.46	10.53	3.71	26.90	25.24	6.44	1.10	1.55	1.62	1.47
G16NL817 x TTN01	70.06	70.58	288.95	146.88	3.33	5.07	8.60	3.72	27.48	23.89	6.41	1.05	1.56	1.42	2.16
G16NL777 x TTN01	70.03	70.31	287.02	148.24	0.61	3.40	4.00	5.27	28.14	26.53	6.38	1.08	1.81	1.69	2.85
G16NL805 x TTN01	69.75	70.34	281.09	146.27	2.33	3.43	4.97	4.29	28.25	25.20	6.36	1.07	2.27	1.50	1.11
G16NL827 x TTN01	70.93	70.71	288.14	149.48	8.79	5.25	13.91	4.82	28.08	24.46	6.36	1.10	1.54	1.46	2.40
G16NL806 x TTN01	69.93	70.53	281.58	147.07	2.43	3.94	4.68	4.01	28.11	25.06	6.34	1.07	2.22	1.52	1.25
G16NL898 x TTN01	69.43	69.64	281.47	143.19	2.53	3.24	4.20	6.07	28.51	25.18	6.33	1.07	1.99	1.67	1.78
G16NL915 x TTN01	71.47	70.93	288.66	148.88	2.58	3.26	6.67	3.81	28.37	24.46	6.33	1.05	1.63	1.51	2.04
G16NL834 x TTN01	71.52	70.90	289.75	148.88	1.99	5.56	7.31	3.88	27.72	24.60	6.33	1.05	1.54	1.55	2.08
G16NL747 x TTN01	70.85	71.03	286.90	147.50	8.11	4.35	12.39	4.55	26.58	24.40	6.31	1.07	1.51	1.59	2.64
Heritability	0.52	0.51	0.54	0.50	0.67	0.60	0.65	0.54	0.53	0.32	0.52	0.46	0.39	0.44	0.42
Mean of selected hybrids	70.33	70.59	289.46	148.83	3.74	4.09	8.11	4.50	28.11	24.82	6.48	1.08	1.78	1.60	2.05
Grand mean	69.53	70.21	285.34	145.22	5.49	3.97	9.33	6.59	28.10	23.28	5.62	1.07	2.09	1.71	1.84
Minimum	65.23	67.09	269.67	131.07	0.00	1.64	2.08	0.00	24.39	16.17	3.16	0.97	0.97	1.38	0.85
Maximum	72.50	72.09	299.34	158.54	20.32	11.06	30.35	41.76	30.46	26.53	6.75	1.18	4.74	2.02	3.75
R^2	0.84	0.79	0.96	0.93	0.73	0.41	0.70	0.62	0.72	0.64	0.89	0.32	0.82	0.78	0.75

AD=Days to mid-pollen, SD=Days to mid-silking, PH-Plant height (centimetres), EH=Ear height (centimetres), SL=Stalk lodging (percentage), RL=Root lodging (percentage), TL= Total lodging (percentage), HC= Husk cover (percentage), NP= number of plants per $6m^2$, EHP= Ears harvested per $6m^2$, GY= Grain yield (t/ha), MSV= Maize streak virus, PLS= *Phaesopharia* leaf spot, RST- Rust, GLS= Grey leaf spot

3.3.2 Cross validation of GEBVs and GEGVs

Table 3.9 shows the GEBVs and GEGVs estimated using a GBLUP model and evaluated through k-fold cross-validation. The average correlation coefficients for GEBVs across the five folds were 0.54 for AD, 0.50 for SD, 0.54 for TL, 0.69 for HC, 0.71 for EHP, and 0.57 for GY. The highest average correlation coefficient was observed for EHP (0.71), followed by HC (0.69), indicating a strong prediction accuracy for these traits. In contrast, the lowest average correlation coefficient was observed for SD (0.50). The GEGVs showed similar patterns of correlation coefficients across the traits, with average values of 0.56 for AD, 0.54 for SD, 0.54 for TL, 0.67 for HC, 0.68 for EHP, and 0.55 for GY. GEGVs tended to have slightly higher correlation coefficients than GEBVs for most traits, except for HC and EHP, where GEBVs had higher correlation coefficients. Both GEBVs and GEGVs obtained are effective in predicting the genetic merit of individuals for the traits.

Table 3.9 K-fold cross-validation for the GBLUP model for agronomic traits.

GEBVs						
	AD	SD	TL	HC	EHP	GY
Folds						
1	0.48	0.47	0.56	0.71	0.68	0.60
2	0.54	0.45	0.48	0.68	0.72	0.55
3	0.61	0.51	0.52	0.67	0.71	0.56
4	0.52	0.52	0.55	0.73	0.73	0.58
5	0.53	0.54	0.58	0.66	0.71	0.54
Average	0.54	0.50	0.54	0.69	0.71	0.57
GEGVs						
1	0.57	0.50	0.54	0.68	0.61	0.62
2	0.54	0.51	0.52	0.70	0.69	0.51
3	0.56	0.58	0.51	0.67	0.70	0.56
4	0.57	0.55	0.50	0.65	0.67	0.54
5	0.58	0.57	0.61	0.64	0.73	0.53
Average	0.56	0.54	0.54	0.67	0.68	0.55

GEBVs=genetic estimated breeding values, GEGVs=genetic estimated genetic values, AD=days to mid-pollen, SD=days to mid silking, TL=total lodging (percentage), HC=husk cover (percentage), EHP=ears harvested per 6m2, GY=grain yield (t/ha)

3.3.3 Ecovalence for yield and component traits and foliar diseases reaction

Ecovalence is a measure of genotypes' stability across environments. Low ecovalence values are associated with stable expression of the trait of interest. A summary of the best-selected 20 maize hybrids and their ecovalence for agronomic traits and foliar disease rating are presented in Table 3.9. The most stable hybrids with low ecovalence values for reduced days to mid-pollen were G16NL684 x TTN01 (0.19), G16NL915 x TTN01 (0.21), G17NL237 x TTN01 (0.32), G17NL544 x TTN01 (0.40) and G16NL898 x TTN01 (0.50). Days to mid-silking values ranged from 0.05 (G16NL684 x TTN01) to 3.85 (G16NL817 x TTN01). The hybrid designated G16NL684 x TTN01 (0.05) had the lowest ecovalence, followed by SC727 (0.30), G17NL237 x TTN01 (0.40), G16NL806 x TTN01 (0.43) and G16NL816 x TTN01 (0.46). The ecovalence values varied from 0.93 (G16NL805 x TTN01) to 24.73 (G16NL777 x TTN01) for plant height. The following hybrids recorded good stability for plant height: G16NL805 x TTN01 (0.93), G16NL817 x TTN01 (1.82), G17NL544 x TTN01 (2.68), G16NL860 x TTN01 (3.41) and G16NL827 x TTN01 (3.72). Good and stable ear height was recorded for G16NL747 x TTN01 with ecovalence of 0.27, followed by G16NL721 x TTN01 (2.43), G16NL806 x TTN01 (2.64), G16NL898 x TTN01 (3.47) and G17NL544 x TTN01 (3.62) among the top hybrids. For stem lodging tolerance, G16NL915 x TTN01 with ecovalence of 0.16, followed by G16NL721 x TTN01 (0.21), G16NL860 x TTN01 (0.50) and G17NL544 x TTN01 (0.59) had the lowest values, making them the most desirable selections. The hybrid designated as G16NL805 x TTN01 with ecovalence of 0.43, was rated the best for root lodging, followed by G16NL915 x TTN01 (0.92), G17NL544 x TTN01 (1.40), G16NL684 x TTN01 (1.54) and G16NL834 x TTN01 (1.62). The following hybrids had low ecovalence for total lodging tolerance: G16NL915 x TTN01 (0.74), G17NL544 x TTN01 (0.87), G16NL834 x TTN01 (1.77), G16NL817 x TTN01 (2.25) and G16NL860 x TTN01(2.57) in a desirable direction. Low ecovalence for husk cover were estimated for G16NL679 x TTN01 with 0.75, followed by G16NL806 x TTN01 (1.05), G16NL805 x TTN01 (1.29), G16NL816 x TTN01 (1.44) and G16NL834 x TTN01 (2.03). The following hybrids were desirable and expressed low ecovalence for the number of plants per $6m^2$: G16NL827 x TTN01 (0.78), G16NL805 x TTN01 (0.95), G16NL684 x TTN01 (1.21), G16NL721 x TTN01 (1.54) and G16NL806 x TTN01 (1.64). The hybrids with low ecovalence for the number of ears per $6m^2$ were G16NL834 x TTN01 (0.33), G16NL806 x TTN01 (0.63), G16NL816 x TTN01 (0.64), SC727 (1.24) and G16NL679 x TTN01 (1.56). Ecovalnce for grain yield varied from 0.46 (G16NL721 x TTN01) to (G16NL679 x TTN01). Top and stable yielding hybrids were G16NL721 x TTN01 (0.46), G16NL777 x TTN01 (0.50), G16NL805 x TTN01 (0.51), G17NL544 x TTN01 (0.61) and G16NL747 x TTN01 (0.62) based on low values of ecovalence. Ecovalence for maize streak virus rating varied from 0.00 (G17NL544 x TTN01) to 0.45 (G16NL679 x TTN01). Similarly, low *Phaesopharia* leaf spot values were recorded for G16NL805 x TTN01 (0.02), followed G16NL860 x TTN01 (0.14), G16NL827 x TTN01 (0.17), G16NL819 x TTN01 (0.26) and G16NL806 x TTN01 (0.27) making them the most stable and resistant hybrids. Across the environments the mean rust severity was < 1.00. Low ecovalence for GLS were computed for the top 20 hybrids. Ecovalnce for GLS reading ranged from 0.11 (G17NL237 x TTN01) and 0.98 (G16NL816 x TTN01). The mean traits response of the selected hybrids was higher than the grand means for the most assessed traits. The coefficient of determination value was relatively high (>60%) for most of the assessed traits, except for RL (45%) and MSV (30%)

Table 3.10 The ecovalence for the best-selected 20 maize hybrids based on agronomic traits and foliar disease rating.

	Agronomic traits											Disease parameters			
Crosses	**AD**	**SD**	**PH**	**EH**	**SL**	**RL**	**TL**	**HC**	**NP**	**EHP**	**GY**	**MSV**	**PLS**	**RST**	**GLS**
G17NL544 x TTN01	0.40	0.49	2.68	3.62	0.59	1.40	0.87	6.26	3.31		0.61	0.00	0.65	0.14	0.23
SC659	2.47	2.56	11.90	8.69	5.29	4.44	7.35		4.24	4.99	0.89		0.87	0.52	0.61
G16NL721 x TTN01	1.78	1.57	15.23	2.43	0.21	6.93	4.31		1.54	2.89	0.46	0.01	0.83	0.55	0.54
G16NL819 x TTN01	1.64	1.90	7.93	6.90	4.46	3.83	4.90	2.23	5.37	3.19	0.78		0.26	0.88	0.16
G17NL237 x TTN01	0.32	0.40	10.34	11.58	4.70	6.56	4.22	8.05	6.82	6.72	0.81		0.30	0.17	0.11
G17NL642 x TTN01	0.84	2.53	8.22	5.75	2.12	7.90	5.79	2.34	1.68	3.75	0.76	0.30	0.56	0.29	0.39
G16NL860 x TTN01	1.14	1.66	3.41	8.90	0.50	2.75	2.57	3.32	1.85	3.76	1.04	0.03	0.14	0.02	0.72
SC727	0.73	0.30	4.91	4.28	2.04	3.91	5.28	11.15	2.14	1.24	1.29	0.37	1.81	0.49	0.28
G16NL816 x TTN01	0.90	0.46	12.51	8.98	3.28	8.39	9.30	1.44	2.69	0.64	1.02		0.49	0.22	0.98
G16NL684 x TTN01	0.19	0.05	3.97	4.97	2.48	1.54	3.65	3.79	1.21	5.31	1.28	0.01	0.59	0.37	0.39
G16NL679 x TTN01	1.00	1.54	10.54	5.56	3.88	2.05	8.60	0.78	5.80	1.56	1.66	0.45	0.40	0.35	0.41
G16NL817 x TTN01	2.98	3.85	1.82	3.62	1.54	2.73	2.25	3.40	2.49	2.83	0.83		0.93	0.16	0.90
G16NL777 x TTN01	1.14	1.02	24.73	12.25	2.51	9.59	9.72	6.24	2.23	6.62	0.50		0.59	0.41	0.69
G16NL805 x TTN01	0.95	1.75	0.93	3.71	9.64	0.43	12.21	1.29	0.95	1.95	0.51		0.02	0.49	0.47
G16NL827 x TTN01	0.89	0.57	3.72	6.51	2.23	3.91	2.60	4.92	0.78	4.76	0.77	0.39	0.17	0.97	0.44
G16NL806 x TTN01	0.98	0.43	11.45	2.64	8.15	3.91	9.35	1.05	1.64	0.63	1.41		0.27	0.46	0.18
G16NL898 x TTN01	0.50	0.97	10.23	3.47				5.13	2.51	5.89	0.76	0.09	0.61		0.39
G16NL915 x TTN01	0.21	0.83	10.17	4.24	0.16	0.92	0.74	4.48	1.93		0.87	0.12			0.19
G16NL834 x TTN01	1.02	0.62	10.05	8.01	2.65	1.62	1.77	2.03	1.85	0.33	1.16	0.05	0.60	0.38	0.44
G16NL747 x TTN01	1.33	1.24	7.36	0.27	3.07	3.18	3.31		2.43	2.97	0.62				
Mean of selected hybrids	1.07	1.24	8.60	5.82	3.13	4.00	5.20	3.99	2.67	3.34	0.90	0.17	0.56	0.40	0.45
Grand mean	0.96	1.08	8.95	6.85	3.10	3.51	4.40	3.51	3.23	3.30	0.82	0.10	0.44	0.27	0.33
Minimum	0.00	0.00	0.04	0.03	0.05	0.01	0.02	0.01	0.03	0.03	0.06	0.00	0.00	0.00	0.00
Maximum	10.28	10.75	42.21	46.04	29.28	32.06	32.42	24.00	18.32	10.37	3.22	0.88	3.05	1.27	2.43
R^2	0.82	0.76	0.92	0.91	0.74	0.45	0.67	0.65	0.78	0.61	0.82	0.30	0.77	0.73	0.74

AD=Days to mid-pollen; SD=Days to mid silking; PH= Plant height (centimetres); EH=Ear height (centimetres); SL=Stem lodging (percentage); RL=Root lodging (percentage); TL=Total lodging (percentage); HC=Husk cover (percentage); NP=Number of plants per $6m^2$; EHP=number of ears harvested per $6m^2$; GY=Grain yield (t/ha); MSV=Maize streak virus; PLS=*Phaesopharia* leaf spot; RST=Rust; GLS=Grey leaf spot; R^2=Coefficient of determination

Figure 3.1 presents the relationship between the GEGVs and ecovalence for grain yield for the top-performing 20 single cross maize hybrids. Hybrids at quadrant IV are ideal, exhibiting high GEGVs and low ecovalence values. The following hybrids situated in quadrant IV had the highest grain yield and lowest ecovalence values, respectively: G17NL544 x TTN01 (6.75 t/ha and 0.61), SC659 (6.75 t/ha and 0.89) and G16NL721 x TTN01 (6.60 t/ha and 0.46), making them the most desirable selections. Other selected hybrids combining good GEGVs and low ecovalence values included G16NL819 x TTN01 (6.59 t/ha and 0.78), G17NL642 x TTN01 (6.57t/ha and 0.76), and G17NL237 x TTN01 (6.59 t/ha and 0.81) (quadrant IV). Crosses G16NL860 x TTN01 (6.55 t/ha and 1.04), SC727 (6.55 t/ha and 1.29), G16NL816 x TTN01 (6.55 t/ha and 1.02) and G16NL684 x TTN01 (6.52 t/ha and 1.28), in that order (quadrant I), had high yield and good ecovalence values. The following hybrids (quadrant III) had comparatively lower grain yield GEGVs but recorded low ecovalence: G16NL777 x TTN01 (6.38t/ha and 0.50), G16NL805 x TTN01 (6.36 t/ha and 0.51), G16NL827 x TTN01 (6.36 t/ha and 0.77) and G16NL817 x TTN01(6.41t/ha and 0.83), suggesting that the hybrids have good grain yield stability across environments. Among the top-performing hybrids, G16NL679 x TTN01 (quadrant II) had a mean grain yield of 6.44 t/ha and the highest ecovalence (1.66), making it the least stable variety.

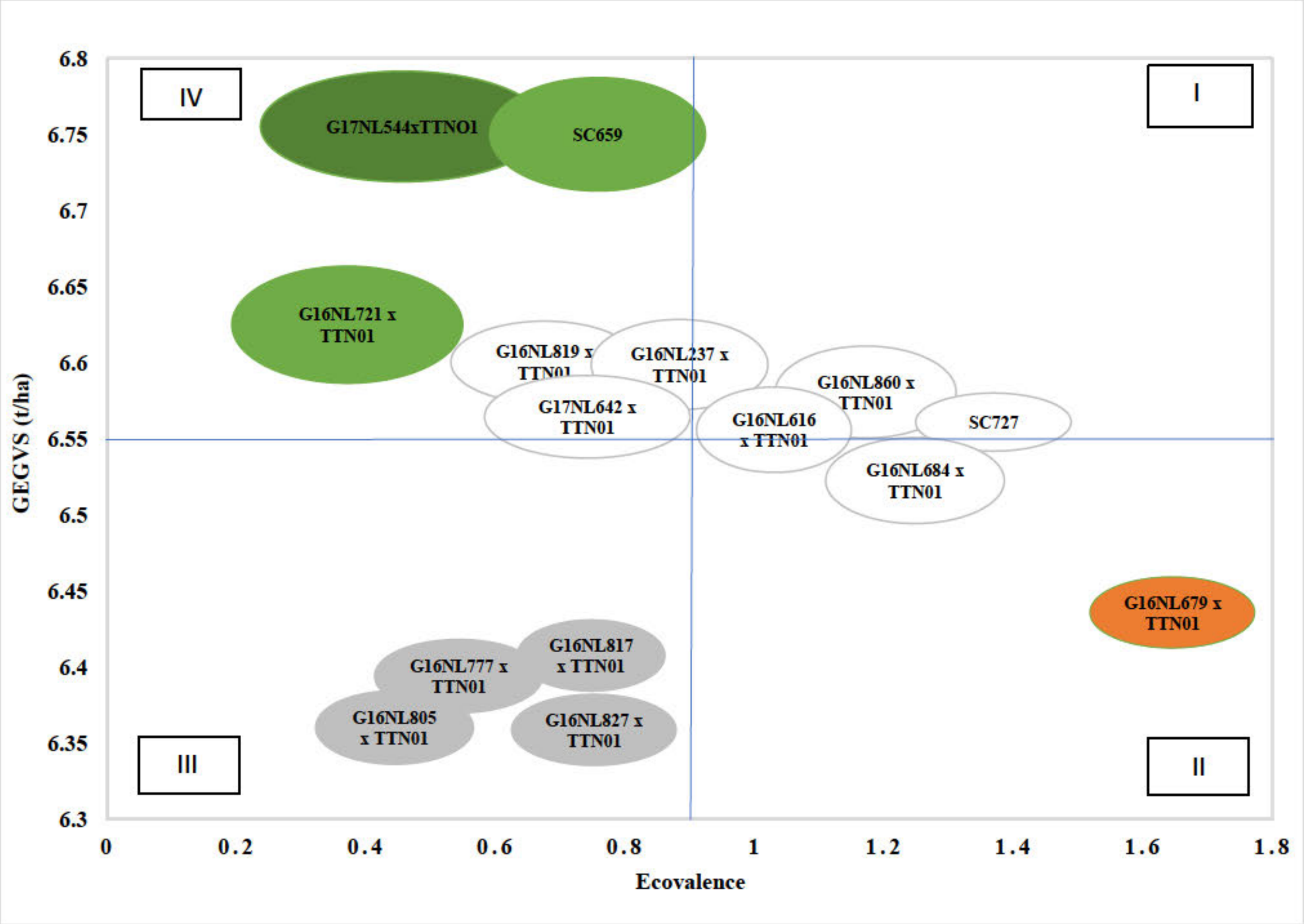

Figure 3.1 The relationship between genomic estimated genetic values (GEGVs) and ecovalence across environments for grain yield (t/ha) of the top-performing 20 maize hybrids. Note that various colours denote the level of desirability of the hybrids according to their GEGVs (t/ha) and ecovalence. Green=most desirable and orange=least desirable.

3.3.4 Influence of the testing environments on selection

The test environments had differential effects on hybrid performance when compared using the genomic estimated genetic values. The positive and negative effects on grain yield performance of the assessed maize hybrids are presented in Table 3.10. The testing location Rattray Arnold Research Station positively affected the grain yield response of genotypes. The highest GEBVs effect for GY (4.55 t/ha) was recorded during the field trials conducted at this location in 2018/2019 season. Furthermore, the following positive GEBVs effects were noted: 4.26t/ha, 4.17 t/ha, 3.97 t/ha, 3.86 t/ha and 3.85 t/ha in 2018/2019, 2019/2020, 2018/2019, 2021/2022 and 2021/2022 seasons, respectively, at this site. This suggested that it was an ideal environment for genotype evaluation, selection and seed production. Gwenhoro (3.84 t/ha in the 2019/2020 season) and Stapleford (3.66 t/ha in the 2019/2020 season) contributed to positive effects on GEBVs of grain yield performance of the evaluated maize hybrids. Conversely, the Kadoma Research Centre negatively affected the grain yield response of the assessed maize hybrids. This location recorded the highest negative effect for GY (-5.7 t/ha) in the 2020/21 season. Additional negative GEBVs effects were recorded at KRC at -5.02 t/ha, -4.24 t/ha and -3.93 t/ha in the 2020/2021, 2018/2019 and 2019/2020 seasons, respectively. The testing locations such as Chakari (-4.79 t/ha in the 2020/2021 season), University of Zimbabwe farm (-4.03 in the 2020/2021 season, -3.53 in the 2019/2020 and -3.48 t/ha in the 2020/2021) and Mvurwi (-3.47 t/ha in the 2018/2019) conferred negative GEBVs effects on GY performance of the assessed maize hybrids, suggesting that these environments are relatively unsuitable for hybrid selection and recommendation. The repeatability values ranged from 9% (RARS in the 2021/22 season) to 81% (RARS in 2019/2020). Standard deviation and standard error values were low for all the testing environments. The coefficient of determination was relatively high (>97%) for all the growing test seasons and locations, explaining the contribution of the testing environments on GEBVs effects.

Table 3.11 The effects of the test environments on grain yield genomic estimated genetic values (GEGVs) of maize hybrids evaluated in five years and seven locations in Zimbabwe.

Year	Location	GEBV effect	Repeatability	Std Dev	Std Error	R^2
2018/2019	RARS	4.55	0.19	1.12	0.40	0.98
2018/2019	RARS	4.26	0.15	1.01	0.39	0.98
2019/2020	RARS	4.17	0.35	0.85	0.32	0.99
2018/2019	RARS	3.97	0.10	1.95	0.39	0.98
2021/2022	RARS	3.86	0.12	1.50	0.48	0.97
2021/2022	RARS	3.85	0.09	1.81	0.45	0.97
2019/2020	GWEN	3.84	0.52	1.09	0.31	0.99
2019/2020	RARS	3.77	0.81	0.53	0.29	0.99
2019/2020	STAP	3.66	0.15	1.21	0.36	0.98
2019/2020	RARS	3.49	0.29	1.00	0.35	0.98
2020/2021	KRC	-5.27	0.81	0.29	0.29	0.99
2020/2021	KRC	-5.02	0.60	0.53	0.30	0.99
2020/2021	CHAK	-4.79	0.67	0.40	0.31	0.99
2018/2019	KRC	-4.24	0.55	0.47	0.30	0.99
2021/2022	KRC	-4.10	0.52	0.47	0.30	0.99
2020/2021	UZ FARM	-4.03	0.67	0.43	0.30	0.99
2019/2020	KRC	-3.93	0.32	0.75	0.35	0.98
2019/2020	UZ FARM	-3.53	0.32	0.92	0.35	0.98
2020/2021	UZ FARM	-3.48	0.42	0.63	0.31	0.99
2018/2019	MV	-3.47	0.21	1.00	0.35	0.98

GEBV=Genomic estimated breeding value; Std Error =Standard error; Std Dev = Standard deviation; R^2=Coefficient of determination, * See Table 3.1 for the codes of test locations.

3.4 Discussion

3.4.1 Selection of best-performing inbred lines using GEBVs and GEGVs

The top-performing lines were selected from each heterotic group (groups 1 and 2) based on their desirable GEBVs and GEGVs using the GBLUP model. Based on the two genomic parameters, the study identified contrasting lines for agronomic traits such as reduced days to mid-pollen, days to mid-silking, anthesis to silking interval, plant height, ear height, tolerant to root lodging, stem lodging, total lodging, foliar diseases, with better husk cover, a higher number of plants per 6m^2, ears harvested per 6m^2, and ears per plant (Tables 3.4, 3.5, 3.6 and 3.7). These results enabled the selection of genetically contrasting inbred lines for hybrid cultivar development with enhanced heterozygosity. The GEBVs have been used to predict various agronomic traits in maize breeding, such as plant and ear height, lodging tolerance, husk cover, ear length, ear diameter, number of rows per cob, number of kernels per cob, 1000 kernel weight and grain yield (Liu et al., 2018; Yang et al., 2020).

Using GEGVs, lines from different heterotic groups can be intercrossed to develop superior hybrids. The study identified promising lines with low GEBVs and GEGVs for days to mid-pollen and days to mid-silking, namely G15NL310, G15NL304, 15AG143, G18NL248, G16NL724, G17NL642, G16NL898 and G17NL642. These lines are the best candidates for hybrid breeding targeting early flowering and maturity. Early maturation is desirable for drought escape and mitigating drought stress under rainfed maize production. Araus et al. (2012) and Badu-Apraku et al. (2013) reported that earliness has often been prioritised to generate maize varieties that can withstand drought. Lines with low and desirable GEBVs and GEGVs for anthesis to silking intervals, such as CTL03, 15AG162, 15AG163, GS-PL07 and 15AG111 are suitable for drought tolerance breeding. Reduced anthesis to silking interval is vital for breeding high-yielding cultivars under drought. Early silking genotypes maintain silk exertion and ear growth, which is necessary for ensuring flower syncing and good pollination (Bolaños & Edmeades, 1996). The following inbred lines, G15NL310, G18NL248, G16NL805, G16NL777 and G16NL89, were selected for their low GEBVs and GEGVs for plant height and ear height. The lines are suitable to breed superior and shorter maize genotypes with good lodging tolerance. Reduced plant and ear height are vital in lodging tolerance, notably under fertile and waterlogging soil conditions. Susceptibility to stem and root lodging and poor husk cover negatively impact maize production and yield gains. The study identified the following lines for their lodging tolerance: G16NL806, G16NL721, G16NL819, G17NL200 and CTL03. Lines GS-PL26, G16NL99, CTL22, G16NL777 and G16NL100 had good husk cover. These lines are suitable for lodging tolerance breeding integrating good husk cover. The present findings agree with Covarrubias-Pazaran et al. (2021) and Loskutov (2021), who reported that GEBVs-assisted genomic selection enhances the accuracy of selection for agronomic traits during line development. GEBVs-assisted genomic selection enhances the pace and precision of selection in plant breeding programs.

From heterotic groups 1 and 2, the study identified CTL03, GS-PL07, 15AG162W, G17NL544, G16NL721 and G16NL860 based on their high GEBVs and GEGVs for grain yield (Tables 3.4, 3.5, 3.6 and 3.7). These lines are suitable for developing high-yielding hybrids. Contrasting lines from the same heterotic groups with high GEBVs can be selected to create new and improved breeding populations. Lines with high GEGVs from different heterotic groups create superior hybrids. These findings agree with Massman et al. (2013) and Beyene et al. (2015), who reported that GS has resulted in the speed breeding of inbred lines with high general and specific combining abilities, allowing rapid hybrid variety development. Bernardo (2014) reported the rapid development of elite inbred lines using genomic prediction. The following lines: 15AG162, 15AG163, 15AG111, CTL22, G16NL816, G16NL804, G17NL642 and G16NL805 were identified for their tolerance to the major foliar diseases assessed in the study, including MSV, GLS, PLS and RST. The lines are suitable for resistance breeding programs. Foliar diseases are the main yield-limiting factors in maize production in warm and humid tropics. Major diseases such as grey leaf spot, northern corn leaf blight and maize streak virus may cause yield losses of 50%, 30-50%, and 100%, respectively (Ward et al., 1997; Gregory, 2004; Shepherd et al., 2010). Previous studies indicated that GEBVs have been beneficial in identifying superior maize inbred lines with resistance to maize diseases, resulting in the subsequent development of resistant varieties (Rice and Lipka 2021).

GEBVs and GEGVs complement or replace trait phenotyping, leading to a significant restructuring of the existing breeding programs (Clark et al. 2012; Hickey et al. 2014). Various studies reported that GEBVs and GEGVs for agronomic traits enhance selection efficiency in plant breeding programs (Hickey et al., 2014; Wang et al., 2020; Auinger et al., 2021) through shortening of the breeding cycle and competitive hybrids development. GS reduces phenotyping and enables parental selection based on GEBVs and GEGVs (Schrag et al., 2008; Albrecht et al., 2011; Rutkoski et al., 2011; Jiang & Reif, 2015; Martini et al., 2016). Genomic-predicted selection is known by the application of "untested parents", given that the inbred parents are selected without field testing. The use of genome-profiled parents accelerates the breeding cycle, resulting in faster genetic advancement and precision hybrid development.

The present study recorded moderate broad-sense heritability values (30-60%) for most of the assessed traits (Tables 3.4, 3.5, 3.6 and 3.7). Trait heritability impacts GS prediction accuracy in maize populations (Daetwyler et al., 2008; De Roos et al., 2009; Isidro et al., 2015). Higher heritability improves prediction accuracy (Zhang et al., 2017). The results agree with De Roos et al. (2009), who reported significant correlations between trait-environment combinations' heritability and prediction accuracy, with highly heritable combinations exhibiting higher prediction values. Higher heritability estimates resulted in higher prediction accuracy for grain yield (Liu et al., 2018).

3.4.2 Selection of maize hybrids using GEGVs

The study identified maize hybrids denoted as G17NL544 x TTN01, G16NL721 x TTN01, G16NL819 x TTN01, G17NL237 x TTN01 and G17NL642 x TTN01 based on high GEGVs for assessed agronomic traits and foliar diseases (Table 3.8). This lineage of the top hybrids followed the top selected lines (Tables 3.4, 3.5, 3.6 and 3.7) except line G17NL237. The findings suggested that lines with the best GEBVs and GEGVs confer top-performing hybrids. The selected single cross experimental hybrids are recommended for commercialization or developing three-way or double cross hybrids. The present findings agree with Cooper et al. (2014), who reported the development and commercialization of 78 AQUAmax® hybrids in the USA using GS. The hybrids had significantly higher yields under favourable and drought-stressed conditions. Only two commercial checks hybrids (SC659 and SC727) were par with the new hybrids. Beyene et al. (2019) reported that hybrid performance can be predicted using genomic prediction using lines with high GEBVs and GEGVs. The top 15% of hybrids which were developed using GS had 21% higher grain yield under well-watered and 52% under managed drought stress conditions than the mean of the commercial checks (Beyene et al., 2019). The use of GS to select superior inbred lines and develop novel hybrids is consistent with previous findings, who have successfully selected high-performing maize germplasm with drought tolerance (Zhang et al., 2015; Shikha et al., 2017) and key agronomic traits, including kernel numbers per row, row numbers per cob and grain yield (Shikha et al., 2017).

3.4.3 Model evaluation using cross-validation

The average prediction accuracy was computed across all k folds and then compared to the accuracy of the model trained on the entire training set. Cross-validation was employed to evaluate the robustness of the genomic selection model and its capacity to predict genomic estimated breeding values (GEBVs) and genomic estimated genetic values (GEGVs) for new data. This approach aids in identifying the most promising inbred lines for genetic advancement and commercial hybrid development (Crossa et al., 2013; Zhang et al., 2015). The GEBVs and GEGVs exhibited moderate to high prediction accuracy for most traits (Table 3.9), with average correlation coefficients ranging from 0.50 to 0.71. These findings align with Crossa et al. (2013), who reported prediction accuracies between 0.51 and 0.59 in maize breeding populations using the GBLUP model. Among the traits, EHP and HC demonstrated the highest prediction accuracy, while SD showed the lowest. Generally, GEGVs outperformed GEBVs for most traits, with the exceptions of HC and EHP. These results indicate that both GEBVs and GEGVs are valuable for predicting genetic merit, though GEGVs may have a slight advantage in most cases. The moderate prediction accuracy for SD indicates a need for further improvements in this trait. Overall, these findings have significant implications for the application of genomic selection in breeding programs and underscore the necessity for continued research to enhance prediction accuracy.

3.4.4 Stability of maize grain yield and components against ecovalence values

Identifying high-yielding and stable cultivars is crucial in plant breeding programs to enhance and sustain crop production and productivity (Ajeesh Krishma et al., 2021; Prasanna et al., 2021). Eyhérabide et al. (2016) defined the term “stability” as the ability of a specific genotype to perform consistently through a range of environmental conditions. Ecovalence (*Wi*) is a statistical metric that was introduced by Wricke (1962) and refers to the contribution of each genotype to the G x E interaction sum of squares. A low *Wi* value indicates the stability of genotypes for a particular trait. *Wi* indicates the deviation from the mean in all contexts. Ecovalence measures the contribution of a genotype to the G x E interaction, and a genotype with zero ecovalence is regarded as the most stable and can be recommended for diverse environmental conditions (Becker and Léon, 1988). Superior cultivars exhibit lower ecovalence values (Lin and Binns, 1988). This is corroborated with by Huehn (1990), who alluded that the stability rank gravitates towards zero when a hybrid attains its maximum stability. A lower ecovalence (0.19) for days to mid-pollen for the new hybrid G16NL684 x TTN01 indicates that the hybrid maintains stability across diverse environments. The lowest ecovalence and GEGVs, respectively, for PH were recorded for G16NL805 x TTN01 (0.93, 281.09 cm), G16NL817 x TTN01 (1.82, 288.95 cm) and G17NL544 x TTN01 (2.68, 290.26 cm) suggesting their stability in maintaining plant height across a range of environments.

Breeders and growers prefer crop cultivars with high mean yields under different environments (Hallauer & Miranda, 1988). Previous studies reported high grain yield and stable maize hybrids (Cooper et al., 2014; Ibrahim, 2015; Beyene et al., 2019). Low ecovalence and high GEGVs for grain yield, in that order, were recorded for the following hybrids: G17NL544 x TTN01 (0.61, 6.75 t/ha), G16NL721 x TTN01(0.46, 6.60 t/ha) and G16NL819 x TTN01 (0.78, 6.59 t/ha). The results indicate the stability and wide adaptability of the hybrids across the test environments. The selected experimental hybrids are recommended for cultivation in the study areas and similar agro-ecologies. Higher ecovalence and grain yield values were recorded for hybrid G16NL679 x TTN01 (1.66, 6.41t/ha), indicating high environmental effects on the yield. This suggests that the new hybrid has specific adaptability and can be recommended for cultivation at Stapleford Research Centre and similar agro-ecologies.

3.4.5 Influence of environments on the selection of maize hybrids

The growing environment plays a significant role in the performance and selection of hybrids. Environmental conditions modulate gene expression, causing genotype × environment interaction (G × E). Understanding the G X E interactions will help to identify genotypes with stable yield performance across different environments (Chapman, 2008). An ideal environment has a high capacity for genotype discrimination and selection. The present study found that the test environments had varying effects on hybrid performance (Table 3.10). The Rattray Arnold Research Station was the most discriminating and representative environment, exhibiting the most significant positive effects on hybrid performance. The Rattray Arnold Research Station was the best environment with suitable temperatures, humidity, and total rainfall, which caused better genotype performance. Gwenhoro (3.84 t/ha) and Stapleford (3.66 t/ha) were two other favourable locations with a positive influence on hybrid selection (Table 3.10). Highly significant environmental effects could be explained by different altitudes, moisture and other stress conditions (Löffler et al., 2005). Hybrid performance was the highest at the mid-altitude (1000 to 1500 m above sea level) and moist environments (800 to 1000 mm rainfall), such as RARS and Stapleford, compared with moisture-stressed environments such as KRC and Chakari.

Aykut Tonk and Tosun (2011) and Wolde et al. (2018) reported that non-discriminating locations had undesirable effects on the performance of maize populations. Test locations that are consistently non-discriminatory provide negative effect values on the genotypes (Yan et al., 2007). Discriminatory power of the test environment can be associated with the prevailing biotic and abiotic factors. Under a non-discriminatory test environment, genotypes fail to express their full potential; thus, this environment will have a confounding effect on the identification of superior genotypes (Yan & Tinker, 2006). The adverse effects of the test environment on hybrid performance could also be attributed to hybrid x moisture stress interactions at the mid-altitude dry environments such as in KRC and Chakari, which experience higher temperatures and shortened growing cycles compared to the mid-altitude moist environments. Long growing periods have been associated with late maturation and high yielding potential (Beck et al., 1990; Pingali and Pandey, 2001). Also, hybrid x season interaction effects rendered different responses of the test hybrids. There is a need to validate the present selections under farmer's field conditions to ascertain the current recommendations.

3.5 Conclusions

Inbred lines with distinct genetic profiles and improved performance are necessary for the pipeline and new hybrid breeding of maize. The study identified 20 high-performing and contrasting lines with the highest GEBVs and GEGVs, each from the two heterotic groups for genetic advancement, combining ability tests and hybrid development. Using the selected inbred lines, 20 high-performing experimental single cross hybrids were developed with high GEGVs for cultivar registration and commercialization, as well as three-way hybrid development. Incorporating genomic prediction into conventional maize breeding programs is essential to identify the genetic identity of candidate inbred lines and develop high-yielding hybrids and product profiles.

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Table S 3.1 Inbred lines used in the study.

Sets	Code x designation	Source	Description
1	CTL01, CTL02, CTL03, CTL04, CTL05, CTL05, CTL07, CTL08, CTL09, CTL10, CTL11, CTL12, CTL13, CTL14, CTL15, CTL16, CTL17, CTL18, CTL19, CTL20, CTL21, G, CTL23, CTL24, CTL25, CTL26	CIMMYT	Heterotic group 1 (N3)
	GS-PL63, GS-PL10, GS-PL58, GS-PL06, 15AG119, 15AG142, 15AG143, 15AG148, 15AG149, 15AG151, 15AG175, GS-PL23, GS-PL52, 15AG104, 15AG176, GS-PL54, 15AG112, 15AG127, 15AG129, 15AG152, GS-PL180, 15AG111, 15AG114, 15AG132, 15AG140, 15AG161, GS-PL66, GS-PL11, GS-PL36, GS-PL01, TTN01, GS-PL44, 15AG110, 15AG123, 15AG164, GS-PL69, 15AG117, 15AG128, 15AG158, 15AG160, 15AG173, GS-PL03, GS-PL59, 15AG131, 15AG137, 15AG147, 15AG154, 15AG155, 15AG159, 15AG165, 15AG167, 15AG174, GS-PL24, 15AG106, 15AG107, 15AG116, 15AG125, 15AG126, 15AG130, 15AG144, 15AG153, 15AG171, GS-PL05, GS-PL07, 15AG121, 15AG124, 15AG157, 15AG168, GS-PL64, GS-PL56, GS-PL55, GS-PL02, 15AG145, 15AG163, 15AG172, 15AG109, 15AG120, 15AG122, 15AG136, 15AG138, 15AG166, 15AG177, GS-PL14, GS-PL40, 15AG113, 15AG115, 15AG150, 15AG156, GS-PL57, GS-PL61, GS-PL04, GS-PL39, 15AG105, 15AG133, 15AG135, 15AG139, 15AG169, 15AG103, 15AG146, 15AG170, GS-PL67, 15AG108, 15AG118, 15AG134, 15AG141, 15AG162, 16AG01, 16AG02, 16AG03, 16AG04, 16AG05, 16AG06, 16AG07, 16AG08, 16AG09, 16AG10	Seed Co	Heterotic group 1 (N3)
	GS-PL62, GS-PL32, 16AG16812, GS-PL28,GS-PL08, GS-PL29,GS-PL13, GS-PL31, GS-PL90 GS-PLS32, GS-PL33, GS-PL34, GS-PL35, GS-PL37, GS-PL38, GS-PL190, GS-PL191, G 16AG16786, 16AG16788, 16AG16791, 16AG16792, 16AG16804, 16AG16816, GS-PL35, GS-PL30, 16AG16798, 16AG16815, GS-PL60, 16AG16782, 16AG16814, GS-PL25, GS-PL71, 16AG16784, 16AG16795, GS-PL92, GS-PL09, 16AG16794, 16AG180, 16AG181, 16AG16790, 16AG16802, 16AG16806, 16AG16811, GS-PL27, GS-PL15, 16AG178, 16AG16803, 16AG16807, GS-PL1, 16AG16781, 16AG16783, 16AG16789, 16AG16796, 16AG16797, 16AG16801, 16AG16805, 16AG181, 16AG16800, 16AG16785, 16AG16809, 16AG16817, GS-PL26, 16AG16793, 16AG16799, 16AG16808, 16AG179, 16AG16787, 16AG16810, 16AG16813, GS-PL46, GS-PL47, GS-PL180, GS-PL181, GS-PL182, GS-PL183, GS-PL184, GS-PL185, GS-PL186, GS-PL187, GS-PL188	Seed Co	Heterotic group 2 (SC)
2	G15NL01 - G15NL12; G15NL14 - G15NL20; G15NL22 - G15NL27; G15NL29 - G15NL36; G15NL281; G15NL283 - G15NL314; G15NL316 - G15NL321; G15NL323 - G15NL334; G15NL336 - G15NL342; G15NL344 - G15NL357; G15NL359 - G15NL365; G15NL367 - G15NL370 G16NL37 - G16NL50; G16NL54 - G16NL68; G16NL70 - G16NL74; G16NL77 - G16NL83; G16NL85 - G16NL99; G16NL100 - G16NL109; G16NL112 - G16NL138; G16NL141 - G16NL145; G16NL147 - G16NL153; G16NL155, G16NL156 GL17NL157 - G17NL170; G17NL172 - G17NL180; G17NL182 - G17NL226; G17NL228 - G17NL239; G17NL241 - G17NL242 G18NL206, G18NL240 - G18NL246; G18NL248 - G18NL254; G18NL257 - G18NL267; G18NL269 - G18NL271; G18NL273 - G18NL280	Seed Co	Heterotic group 1 (N3)
	G16NL677 - G16NL813; G16NL815 - GL16NL850; GL16NL852 - GL16NL921 G17NL373 - G17NL467; G17NL469 - G17NL484; G17NL486 - G17NL664 G18NL665 - G18NL676	Seed Co	Heterotic group 2 (SC)

CIMMYT= International Centre for Maize and Wheat Improvement

CHAPTER FOUR

Performance of Single-Cross Maize Hybrids Selected Using Genomic Predictions for Yield and Yield Components

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Abstract

Genomic-assisted breeding has the potential to enhance gains in yield and yield components through precision and speed breeding. Single cross maize hybrids are high-performing and resilient to diverse growing conditions, which can bolster maize production and productivity. The objective of this study was to assess the gains in yield and yield components among single cross maize hybrids selected through genomic prediction across representative locations to guide breeding and production. Thirty hybrids were developed from inbred lines with the highest predicted genomic estimated genetic values (GEGVs) for grain yield and associated traits. The 30 hybrids and six commercial single cross check hybrids were evaluated in seven locations, four in Zimbabwe and three in Zambia. A combined analysis of variance revealed significant variation among the hybrids for the assessed 11 quantitative traits. Significant yield gains were realized over the mean of checks (at 13.09%), mean of the population (10.83%) and mean of best check (1.47%). Moderate to high broad-sense heritability (50 to 94%) and genetic advance were recorded for most of the assessed traits, indicating the success of selection assisted by genomic predictions. The study identified three best single cross hybrids (i.e., CTL03 x G16NL721, CTL03 x G17NL544 and GS-PL07 x G17NL544) with high and stable yields and recommended for commercialization. Integrated phenotypic and genomic selection enhances genetic gains and may reduce the breeding cycles and costs of hybrid breeding in maize.

Keywords: *heritability, genetic advance, genetic gain, genetic variation, genomic prediction, single cross*

4.1 Introduction

Maize (*Zea mays* L., 2n = 2x = 20) is valued for food, feed, bioethanol production and as a source of industrial raw material globally (Byerlee, 2020). The discovery of hybrid vigour or heterosis in 1909 enabled the development of hybrid maize in the 1920s in the USA and 1930s in China (Galinat, 1988; Hallauer, 2007). In 1924, the first maize hybrids from varietal crosses were marketed in the USA. In the 1930s, the first successful double-cross hybrids were released, followed by a transition into single-cross hybrids in the 1950s (Hallauer, 2007; Byerlee, 2020). In Africa, the first single cross hybrid of maize (designated as SR52) bred by Alan Rattray was commercialized in 1960. Consequently, SR52 became the foundation for hybrid breeding programs in central, eastern and southern Africa (Derera and Musimwa, 2015).

To date, maize is a staple food crop in many African countries, commonly grown by millions of resource-poor smallholder farmers (Beyene et al., 2015). The total area under maize production is approximately 42 million hectares in Africa, providing an estimated grain yield of 91 million tonnes per annum (FAOSTAT, 2024). Maize productivity in Africa is low (< 2 t/ha) compared to a global mean of 6 t/ha. Several factors contribute to low production and productivity, including the high incidence of abiotic and biotic stresses, socio-economic constraints, and policy issues (Beyene et al., 2015). The major abiotic production constraints include drought and heat stress, flooding, waterlogging, erosion and poor soil health (Santpoort, 2020; Thomas, 2020; Siatwiinda et al., 2021). The major biotic stresses that contribute to significant yield losses or crop failure are plant diseases (e.g., grey leaf spot, *Turcicum* leaf blight, leaf rust, maize streak virus and maize lethal necrosis), parasitic weeds (e.g. *Striga* species), and insect pests (e.g., stalk borer, grain weevils and the fall armyworm) (Cairns et al., 2013; Beyene et al., 2017). The pace and success of modern cultivar development and deployment with resistance to biotic and abiotic stresses are crucial for stable maize production in Africa.

Genomic-assisted breeding has the potential to enhance gains in yield and yield components through precision and speed breeding (Windhausen et al., 2012; Crossa et al., 2014; Varshney et al., 2017; Hickey et al., 2019). Genomic estimated genetic values (GEGVs) are estimated from genetic analysis using several marker systems from which an estimate of the genetic value of a genotype can be derived. The estimates are used to rank and select among genotypes, inbred lines, clones or families (Crossa et al., 2010). Inbred lines with high GEGVs can accelerate the development of superior hybrids in maize and related crops.

High maize productivity has been recorded in the USA, China, Brazil, Argentina, India, Mexico, Ukraine, Indonesia, South Africa, Russian Federation and other major maize producers due to the successful adoption of single cross hybrids (Wang et al., 2011). Single cross maize hybrids (SCHs) are resilient to diverse growing conditions and high-performing, which can bolster maize production and productivity (Joshi and Gautam, 2021). SCHs show maximum heterosis and are highly responsive under productive and high-input conditions. They possess uniform characteristics in terms of plant and ear height, tasseling, silking and pollen shedding, unlike three- and four-way hybrids (Wang et al., 2011; Joshi and Gautam, 2021). The adoption of SCH technology in the US corn belt in the 1960s resulted in a marked rise in maize productivity from 3.5 t/ha in 1960, 5.21 t/ha (1970), 9.68 t/ha (2008) and 11.15 t/ha in 2020, culminating in a relative gain of 114% since 1970 (Rizzo et al., 2022; Ruiz et al., 2023). Further, the adoption of SCHs in Africa led to a 46% increase in yield compared to the popularly grown open-pollinated varieties such as Hickory King (Derera and Musimwa, 2015). The use of SCHs raised maize yields threefold in Africa. Further, the production and maintenance of SCHs is easier than three-way or four-way crosses.

Developing high-yielding SCHs requires genetically contrasting parental lines. Mating designs are used to generate breeding populations that serve as a foundation for selecting and developing varieties, estimating genetic gain and providing information on breeding parents and derived crosses (Acquah, 2012). Various genetic designs, such as the factorial mating design, have been used in plant breeding programs (Muthoni & Shimelis, 2020). All mating designs involve targeted crosses using selected parents to generate half or full-sib progenies. The factorial mating design is widely used in breeding programs when contrasting male and female sets of parental lines are used in genetic analysis and breeding based on full sibs (Wang et al., 2017).

Breeding programs aim to develop and commercialize productive hybrids in a reduced timeline and field phenotyping costs. Consequently, there is a need to integrate genomic prediction in hybrid development to improve breeding efficiency and enhance hybrid development turnaround. Conventional hybrid breeding with pipeline inbred lines takes five to six years to initiate crosses, conduct yield trials and deploy new hybrids. Genomic-enabled prediction can speed up inbred line development, trait integration, and the formation of an increased number of crosses to enhance the response to selection. Genomic prediction using the genomic best linear unbiased prediction (GBLUP) model is promising for enhanced selection efficiency of inbred lines, reduced timeline for hybrid breeding and boosting genetic gains. High-value inbred lines with the best GEGVs for grain yield and component traits were selected (Gunundu et al., unpublished data) for single cross-hybrid development. The selected lines need to be evaluated in hybrid combinations to assess the gains in yield and yield components across representative locations to guide the selection of experimental hybrids for breeding and production. Therefore, the study aimed to assess the gains in yield and yield components among single cross maize hybrids selected through genomic predictions across representative locations for breeding and production.

4.2 Materials and methods

4.2.1 Plant materials

The study used 36 SCHs. The 30 SCHS were developed from crosses using 11 inbred lines with the highest GEGVs predicted using genomic selection (GS) using the genomic best linear unbiased prediction (GBLUP) model. The inbred lines were selected for their contrasting grain yield and component trait responses. The lines were derived from two heterotic groups [N3 (group 1) and SC (group 2)]. The lines were developed for pipeline hybrid breeding, targeting high grain yield, yield components and biotic and abiotic stress tolerance. A total of 11 lines, six from group 1 and five from group 2 were used (Table 4.1). The lines were acquired from the Seed-Co germplasm pool in Zimbabwe.

Table 4.1 Lines with complementary yield and agronomic traits used in single cross-hybrid development and the hybrid checks used in the study

Entry no.	Line designation	Heterotic group	Traits										
			AD	**SD**	**ASI**	**PH**	**EH**	**TL**	**NP**	**EHP**	**EPP**	**MOI**	**GY**
1	CTL03	1	70.40	68.86	0.18	2.87	1.16	8.69	25.67	26.05	1.02	14.55	6.12
2	GS-PL07	1	70.22	70.39	0.47	2.82	1.15	8.68	25.71	25.93	1.00	14.28	5.99
3	15AG162	1	70.10	71.37	0.37	2.82	1.09	8.56	25.32	24.15	0.98	13.23	5.94
4	15AG163	1	69.69	70.40	0.45	2.82	1.08	8.80	25.08	24.40	0.98	15.15	5.90
5	G15NL304	1	67.46	71.23	1.13	2.79	1.06	8.26	25.52	24.12	0.97	15.10	5.90
6	15AG143	1	69.38	68.89	1.05	2.78	1.07	7.72	25.21	24.22	0.97	15.06	5.88
7	G17NL544	2	68.51	70.29	-	2.80	1.41	9.77	28.95	23.71	0.98	14.15	6.81
8	G16NL721	2	68.35	70.30	-	2.79	1.39	3.48	28.73	22.62	0.95	15.02	6.74
9	G16NL642	2	68.18	69.64	-	2.82	1.39	8.85	28.81	24.64	0.95	14.23	6.72
10	G16NL860	2	70.66	71.89	-	2.84	1.42	9.37	28.93	23.88	0.99	15.25	6.66
11	G16NL679	2	69.77	70.75	-	2.83	1.43	8.65	28.48	24.55	0.99	14.25	6.65

Single-cross hybrid checks

Entry no.	Hybrid designation	Source	Adaptation	Maturity
1	SC659	Seed Co	T/ST	Medium-Late
2	SC719	Seed Co	T/ST	Late
3	SC727	Seed Co	T/ST	Late
4	SC729	Seed Co	T/ST	Late
5	PGS65	Klein Karoo	T/ST	Medium -Late
6	PAN7M81	Corteva	T/ST	Late

AD=anthesis date (days to 50% pollen shed), SD=silking date (days to 50% mid-silking), ASI=anthesis-to-silking interval (days), PH=plant height (metres), EH=ear height measure from the ground to the node that bears the upper most ear (metres), TL=total lodging (percentage), NP=number of plants per $6m^2$, EHP=number of ears harvested per $6m^2$, EPP= number of ears per plant, MOI=moisture content at grain harvest (%), GY=grain yield (t/ha), T=tropical, ST=sub-tropical, -=denote not available

4.2.2 Formation of single crosses

The selected inbred lines were crossed with a factorial mating design aiming at the two contrasting heterotic groups as male and female parents. The six lines from group 1 were used as females, while five from group 2 served as male parents with good and extended pollen-shedding ability. The five males were crossed to the six females to produce 30 SCHs. The crossing block was established in Muzarabani/Zimbabwe in winter 2022. The males were stagger planted five days before planting the females, on the same day as the females and five days after the females, to ensure syncing and good pollen coverage for optimal seed production.

4.2.3 Study sites

The study evaluated 30 SCHs, including the six commercial checks. The six commercial SCHs were used as comparative controls. The commercial checks are widely grown varieties in Sub-Saharan Africa owing to their high yield potential, wide adaptability and good agronomic characteristics. The 36 SCHs were evaluated across seven locations: four in Zimbabwe and three in Zambia in the 2022/23 summer season. The description of the study locations in each country are provided in Table 4.2.

Table 4.2 Description of the seven study locations used to evaluate the hybrids in Zimbabwe and Zambia in the 2022/2023 season.

Country	**Location**	**Location code**	**Altitude (masl)**	**Latitude**	**Longitude**	**Total rainfall (mm) during the growing season**
Zimbabwe	Stapleford Research Centre	SRC	1476	17°43'S	30°52'E	820
	Rattray Arnold Research Station	RARS	1360	17°14'S	31°14'E	865
	Kadoma Research Centre	KRC	1149	18°16'S	29°50'E	800
	Agricultural Research Trust	ART	1480	17°44'S	31°03'E	850
Zambia	Mpongwe Research Station	MPRS	1198	13°30'S	28°9'E	1200
	Lusaka West Research Station	LWRS	1219	15°24'S	28°04'E	800
	Mkushi Research Station	MKRS	1254	13°37'S	29°23'E	950

masl - metres above sea level

4.2.4 Experimental design and trial management

The 36 hybrids were evaluated with field trials using a 6 x 6 alpha lattice design with two replications at each location. The plot area consisted of two rows measuring 4m long with inter-row and intra-row spacing of 0.75 m and 0.25 m, respectively. The net plot area was 6m^2 at each site. Three seeds were sown per hill and thinned to one plant one week after emergence to achieve an approximate plant population of 53 333 plants ha^{-1}. Compound fertilizer containing Nitrogen, Phosphorous and Potassium in a ratio of 7:14:7, in that order, was used as a basal application at a rate of 426 kg ha^{-1} in all the testing sites. Ammonium nitrate (34.5 % N) was split-applied as top dressing fertiliser at a rate of 426 kg ha^{-1} at each site. Half of this amount was applied four weeks after planting and the remainder eight weeks after planting. The trials were kept weed-free using a combination of chemical and mechanical weed control. A blend of Integrity® (1 litre ha-1) and Roundup® (3 litres ha-1) were applied as pre-emergence herbicides. Five weeks after germination, hand hoeing was used to remove emerging weeds. Scouting for insect pests such as maize stalk borer (*Busseola fusca*) and fall armyworm (*Spodoptera frugiperda*) was done soon after crop emergence. Dipterex granules were applied four weeks after crop emergency in the leaf whorl of each plant at 2 kg ha^{-1} to control the maize stalk borer. Super dash and Ampligo were alternatively sprayed in the leaf whorl of each plant at two, four and six weeks after crop emergency at 200 – 300 millilitres ha^{-1}

to control the fall armyworm. The experiments were conducted under rain-fed conditions. Supplementary irrigation was provided during dry periods to maintain optimum soil moisture conditions.

4.2.5 Phenotypic data for genomic selection

Phenotypic data were collected on yield and yield components at seven sites, following standard descriptors and procedures (CIMMYT, 1999). The data collected included plant height, ear height, total lodging, number of plants per $6m^2$, grain moisture content at harvest, and grain yield. Flowering parameters were recorded at six sites, such as days to mid-pollen, days to mid-silking, and anthesis-silking interval. Additionally, number of ears harvested per $6m^2$ and number of ears per plant were recorded at five sites (RARS, SRC, KRC, LWRS, and MPRS), due to technical staff limitations. A detailed description of the agronomic traits measured is presented in Table 4.3.

Table 4.3 List and description of traits recorded in the study.

Trait	**Abbreviation**	**Description (units)**
Days-to-mid-pollen	AD	Measured as the number of days from planting date to 50% pollen shed (days).
Days-to-mid-silking	SD	Measured as the number of days from planting date to 50% silk emergence (days).
Anthesis-silking-interval	ASI	Calculated as the difference between days-to-mid-pollen to days-to-mid-silking (days).
Plant height	PH	Determined by randomly selecting and tagging 10 plants from a plot (6 m^2) and measured as the distance between the base of the plant and where the tassel starts to branch (expressed in metres)
Ear height	EH	Determined from 10 randomly selected and tagged plants by measuring the distance from the base of the plant to the node that bears the upper most ear using a measuring tape (expressed in metres).
Total lodging	TL	Determined by adding the number of plants that lodged as a result of root and stalk lodging (expressed in percentage).
Number of plants per $6m^2$	NP	Determined by recording the number of plants per plot (6 m^2) at harvest.
Number of ears harvested per $6m^2$	EHP	Determined by recording the number of ears counted at harvest per $6m^2$.
Number of ears per plant	EPP	Calculated as a ratio of ear count to the number of plants per $6m^2$
Grain moisture content	MOI	Determined by recording grain moisture content after shelling (%). Moisture was adjusted to 12.5%.
Grain yield	GY	Determined by adjusting grain weight to 12.5% moisture content and converting to tonnes per hectare.

Grain yield expressed in t/ha $^{-1}$ was recorded from grain weight per plot adjusted to 12.5% grain moisture following CIMMYT (1999).

$$\mathrm{GY} = \left(\frac{\mathrm{GW}}{\mathrm{NP}}\right) * 10 * \left(\frac{100 - \mathrm{MO}}{87.5}\right)$$

Where;

GY= Grain yield (kg/ha)

GW=Grain weight at harvest (kg/6m^2)

MO= Moisture content (%) of grains at harvest.

87.5= Standard dry matter of grain at 12.5 % as required by maize grain market authorities in Zimbabwe and South Africa

NP= Net plot area (number of rows x intra row spacing x number of stations x inter row spacing).

4.2.6 Data analysis

4.2.6.1 Analysis of variance and mean separation

The collected data were subjected to analysis of variance using the Breeding Management System (BMS) (version 25 (BMSv25)). ANOVA for a single location was performed following Dabholkar (1999):

$$Y_{ilk} = \mu + h_i + r_k + b_l\,(r_k) + e_{ilk}.$$

Where, Y_{ilk} = observed response; μ = grand mean; h_i = effect of the ith hybrid; r_k = effect of the kth replication; $b_l\,(r_k)$ = effect of the $_l$th block nested in the $_k$th replication and e_{ilk} = the error term

After the Bartlet (Arsham and Lovric. 2011) test of homogeneity of variance, a combined analysis of variance was performed based on the formula given by Singh and Chaudhary (1979):

$$Y_{ijkl} = \mu + G_j + E_i + G_j * E + E_i\,(rk)\,(b_l\,) + \varepsilon_{ijkl}$$

Where; Y_{ijkl}=observed response, μ=grand mean G_j=the effect of jth genotype, , E_i=effect of ith environment, G_j*E_i=genotype x Environment interaction $E_i(r_k)(b_l)$=error associated with kth replication in l blocks in ith environment, $\mathcal{E}_{ijkl}$=residual.

4.2.7 Estimation of realised genetic gains

In order to analyse genetic gains of traits assessed from the hybrids, the population mean, means of check hybrids, and the best hybrids were computed across locations. The different methods used to calculate realized genetic gains were designated by RG1, RG2, and RG3, respectively, as described below.

Realised gains were calculated according to Falconer and Mackay (1996):

i. Realised gain (RG1): genetic gains relative to the population mean (trial mean).

$$\mathrm{RG1} = \left(\frac{\mathrm{MS-MP}}{\mathrm{MP}}\right) * 100$$

ii. Realised gain (RG2): genetic gains relative to the mean of the best commercial control hybrid.

$$\mathrm{RG2} = \left(\frac{\mathrm{MS-MBC}}{\mathrm{MBC}}\right) * 100$$

iii. Realised gain (RG3): genetic gains relative to the mean of all commercial control hybrids

$$R\mathrm{G3} = \left(\frac{\mathrm{MS-MC}}{\mathrm{MC}}\right) * 100$$

Where MS= mean of selected hybrids, MP= population mean, MBC= mean of the best commercial check hybrid, and MC= mean of commercial hybrids.

4.2.8 Variance components

Genetic and phenotypic variances were computed according to Wricke & Weber (1986) as follows:

Genotypic variance (σ^2_{g}) = (MSG-MSE/r)

Phenotypic variance $(\sigma^2_p) = \sigma^2 g + \sigma^2 e$

Error variance (V_e) = (MSE/r)

Where MSG, MSE and r are the mean squares of genotypes, mean squares of error and number of replications, respectively and $\sigma^2 e$ = environmental variation

4.2.8.1 Coefficients of variation

The phenotypic and genotypic coefficients of variations were calculated following Singh and Chaudhary (1979) using SAS version 9.3

$$\text{Phenotypic coefficient of variation (PCV, \%)} = \left(\frac{\sqrt{\sigma_p}^2}{\overline{X}}\right) \text{X } 100$$

$$\text{Genotypic coefficient of variation (GCV, \%)} = \left(\frac{\sqrt{\sigma_g}^2}{\overline{x}}\right) \text{X } 100$$

Where,

σ_p^2 = phenotypic variance, σ_g^2 = genotypic variance, $\bar{X}$= Overall mean

Phenotypic and genotypic coefficient of variations values were categorized as low, moderate and high following Sivasubramaniah and Menon (1973), as follows: 0 - 10% = Low, 10 – 20% = Moderate, > 20%= High.

4.2.9 Broad-sense heritability

The broad sense heritability of assessed traits across locations was calculated as described by Hallauer and Miranda (1988):

$$\mathrm{H}^2 = \left[\frac{\sigma^2 g}{\sigma^2 p}\right] \mathrm{X}\ 100$$

Where,

$$\sigma^2 p = \frac{\sigma^2}{re} + \frac{\sigma^2 ge}{e} + \sigma^2 g$$

H^2=broad sense heritability

$\sigma^2 g$ = genotypic variance

σ^2= environment variance

$\sigma^2 ge$= genotype by environment interaction variance

r= number of replications

e= number of sites.

4.2.10 Genetic advance

Genetic advance (GA) and GA as per cent of the mean (GAM) with a selection intensity of 10% were estimated as follows:

The genetic advance was calculated as follows (Falconer and Mackay, 1996):

$$\mathrm{GA} = i\ \sigma_p\ H^2$$

Where,

GA= Genetic advance

i= selection intensity, which has a value of 1.76 at 10% selection pressure

σ_p= phenotypic standard deviation

H^2= broad sense heritability

Genetic advance as percent of mean was estimated as described by Souza et al. (2009) as follows:

$$\mathrm{GA}\ (\%) = \frac{\mathrm{GA}}{\overline{X}}$$

Where,

GA (%) = Genetic advance as percent of mean

GA= Genetic advance

$\overline{\mathrm{X}}$= Grand mean

Genetic advance as a percent of the mean was classified and rated based on the scales given by Johnson et al. (1955) as low (< 10 %), moderate (10-20 %) and high (>20 %).

4.2.11 Correlation analysis

Phenotypic correlations among grain yield and component traits were performed in Genstat 18th edition (Goedhart and Thissen, 2010) to determine the magnitude of relationships between grain yield and component traits using Pearson's correlation analysis.

4.3 Results

4.3.1 Analysis of variance

The summary of the combined analysis of variance for grain yield and associated traits across sites in Zimbabwe and Zambia are presented in Table 4.4. There were significant genotypic differences for most of the recorded traits. Further, significant genotype and location ($p \leq 0.05$) interaction effects were recorded for all the assessed traits.

Table 4.4 Mean square values and significance tests for seven agronomic traits of 30 new hybrids and six commercial hybrids of maize evaluated across seven, six or five locations in Zimbabwe and Zambia.

Source of variation	**df**	**AD^{++}**	**SD^{++}**	**ASI^{++}**	**df**	**PH^{+++}**	**EH^{+++}**	**TL^{+++}**	**NP^{+++}**
Locations (L)	5	2656.57***	2945.80***	157.87***	6	18.39***	0.44***	4757.21***	799.99***
L*Rep	6	26.34	1.26	17.31	7	0.03	0.03	199.61*	8.87
L*Rep*block	60	42.63	6.43***	36.56	70	0.05***	0.06***	196.74***	6.92*
Genotype (G)	35	40.97**	12.60***	39.18**	35	0.13***	0.13***	196.22***	6.78**
L*G	175	34.57**	2.83***	36.34**	210	0.02***	0.03***	161.38***	5.49**
Residual	150	33.31	1.5	39.26	175	0.02	0.02	88.07	4.96
Total	431	66.08	41.32	88.82	503	0.25	0.04	198.57	15.12
Trial statistics									
LSD (5 %)		1.47	1.4	0.79		0.11	0.13	12.77	1.27
CV (%)		11.06	8.72	535.8		16.63	12.8	177.9	12.04
SE		0.39	0.32	0.31		0.02	0.01	0.63	0.17

Source of variation	**df**	**EHP^{+}**	**EPP^{+}**	**df**	**MOI^{+++}**	**GY^{+++}**
L	4	14710.06***	7.83***	6	1150.44***	592.94***
L*Rep	5	34.51	0.02	7	5.667	2.834
L*Rep*block	50	46.03***	0.03***	70	8.53***	2.62**
G	35	39.03***	0.02***	35	14.00***	9.57***
L*G	140	21.05***	0.01***	210	5.34***	2.49***
Residual	125	15.65	0.01	175	3.56	1.6
Total	359	178.57	0.1	503	20.07	10.07
Trial statistics						
LSD (5%)		3.28	0.15		1.93	1.07
CV (%)		51.16	37.51		26.24	37.86
SE		0.79	0.02		0.20	0.14

*; **; *** significance at $P < 0.05$, 0.01 and 0.001, respectively, df=degrees of freedom, AD=anthesis date (days to 50% pollen shed), SD=silking date (days to 50% mid-silking), ASI=anthesis-to-silking interval (days), PH=plant height (metres), EH=ear height measured from the ground to the node that bears the upper most ear (metres), TL=total lodging (percentage), NP=number of plants per 6m^2, EHP=number of ears harvested per 6m^2, EPP=number of ears per plant, MOI=moisture content at grain harvest (%), GY=grain yield (t/ha), LSD=least significance difference at 5% probability level, CV=coefficient of variation (percentage), SE=standard error,$^{+++}$, $^{++}$, $^{+}$ denote evaluations across seven, six and five locations, respectively.

4.3.2 Mean performance of hybrids in Zimbabwe

Table 4.5 summarises the experimental hybrids, hybrid checks and the top 10 hybrids (bold-faced entries) with the best grain yield performance in the Zimbabwe test locations. The earliest days to mid-pollen and days to mid-silking, in that order, were recorded for the following hybrids:15AG162 x G16NL679 (75.57 and 77.53 days) and CTL03 x G16NL544 (75.92 and 77.58 days). The hybrids had comparable values to check hybrid SC659 with 75.84 and 77.10 days, respectively. The following hybrids had reduced anthesis to silking interval: check hybrid SC727 (0.05 days), 15AG162 x G17NL642 (0.10 days), G15NL304 x G17NL544 (0.13 days) and CTL03 x G16NL721 (0.26 days). Plant height varied from 2.27 to 2.91m. The new hybrids with short plant height and ear height were 15AG162 x G16NL679 (2.27m and 1.04 m) and CTL03 x G17NL642 (2.49m and 1.36m), respectively. Commercial checks SC659 (with a plant height of 1.30 m) and SC727 (1.33 m) also exhibited ideal ear heights among the tested hybrids. The new hybrids GSPL07 x G17NL642 (5.67%), G15NL304 x G17NL544 (6.17%) and CTL03 x G17NL544 (6.82%) had low and ideal total lodging tolerance after the check hybrid SC659 (4.56%). Top selections with the highest number of plants per 6m^2 and ears harvested per 6m^2 were GS-PL07 x G17NL544 (33.34 and 31.06), GS-PL07 x G17NL642 (33.31 and 27.57), CTL03 x G17NL544 (32.65 and 28.67) and CTL03 x G16NL721(32.21 and 28.36), respectively. The mean number of ears per plant was 1.00 for the top 10 new hybrids. Hybrid GS-PL07 x G17NL642 (15.35%) recorded the lowest grain moisture content at harvesting, followed by GS-Pl07 x GN17NL544 (17.28%) and CTL03 x G16NL721 (18.20%). Grain yield varied from 8.29 t/ha to 8.91 t/ha for the top 10 selected hybrids. The highest grain yield was expressed by CTL03 x G16NL721 at 8.91 t/ha, followed by 15AG162 x G16NL679 (8.56 t/ha) and G15NL304 x G17NL544 (8.45 t/ha). Check hybrid S727 (with 8.55 t/ha) computed the highest and comparable grain yield among the tested check hybrids. Most assessed traits exhibited moderate to high broad-sense heritability, ranging from 0.35 (TL) to 0.82 (PH), warranting a good response to selection. The top 10 selected hybrids were higher than the grand mean for most traits, except for SD, TL and MOI. Coefficient of variation (CV%) values were low for most of the traits. Moderate to high coefficient of determination (R^2) values were computed for the assessed traits, ranging from 55% (TL) to 94% (PH and GY).

Table 4.5 Mean performance of the experimental and commercial maize hybrids for 11 agronomic traits in Zimbabwe.

Entry	Single cross	AD	SD	ASI	PH	EH	TL	NP	EHP	EPP	MOI	GY
Experimental hybrids												
1	**GS-PL07 x G17NL642**	**77.29**	**79.38**	**2.09**	**2.87**	**1.46**	**5.67**	**33.31**	**27.57**	**0.82**	**15.35**	**8.35**
2	15AG163 x G17NL642	71.61	73.90	2.29	2.49	1.12	7.51	18.75	20.38	0.87	16.33	4.25
3	GS-PL07 x G16NL860	80.45	81.48	1.03	2.56	1.31	8.82	29.47	20.64	0.85	16.43	8.16
4	GS-PL07 x G16NL679	79.59	79.75	0.16	2.05	1.14	8.08	26.07	24.53	0.82	19.84	6.58
5	**CTL03 x G17NL642**	**77.04**	**78.67**	**1.63**	**2.49**	**1.36**	**8.24**	**30.54**	**24.22**	**0.86**	**20.28**	**8.37**
6	15AG162 x G16NL721	80.68	81.03	0.35	2.41	1.17	8.32	31.76	24.72	0.82	19.63	8.16
7	15AG143 x G16NL721	79.97	80.41	0.44	2.69	1.63	6.47	32.44	26.82	0.84	17.60	7.12
8	**G15NL304 x G17NL544**	**79.90**	**80.03**	**0.13**	**2.73**	**1.48**	**6.17**	**30.63**	**22.43**	**0.86**	**19.92**	**8.45**
9	G15NL304 x G17NL642	81.45	83.04	1.59	2.76	1.81	6.37	29.21	25.39	0.84	20.06	8.06
10	**GS-PL07 x G17NL544**	**77.36**	**79.84**	**2.48**	**2.50**	**1.65**	**7.09**	**33.34**	**28.67**	**0.93**	**17.28**	**8.35**
11	15AG163 x G16NL860	78.23	79.04	0.81	2.69	1.44	9.49	30.34	30.90	0.85	18.83	7.49
12	G15NL304 x G16NL860	76.52	76.86	0.34	2.49	1.25	7.21	29.52	24.40	0.86	18.19	7.17
13	15AG143 x G16NL860	76.22	76.75	0.53	2.52	1.22	6.94	30.59	24.93	0.86	16.57	8.18
14	**15AG163 x G16NL721**	**78.12**	**78.78**	**0.66**	**2.99**	**1.67**	**7.17**	**31.66**	**24.35**	**0.84**	**20.46**	**8.27**
15	15AG162 x G17NL544	79.39	80.75	1.36	2.58	1.27	5.11	28.51	23.08	0.85	18.16	7.89
16	**15AG162 x G17NL642**	**78.86**	**78.96**	**0.10**	**2.89**	**1.62**	**7.20**	**28.45**	**21.83**	**0.86**	**18.64**	**8.29**
17	15AG162 x G16NL860	78.05	78.47	0.42	2.43	1.32	9.04	26.13	23.54	0.84	16.96	5.12
18	**CTL03 x G16NL721**	**80.30**	**80.56**	**0.26**	**2.72**	**1.76**	**7.05**	**32.21**	**28.36**	**0.87**	**18.20**	**8.91**
19	G15NL304 x G16NL721	77.34	78.22	0.88	2.62	1.28	4.01	30.39	24.57	0.88	20.21	8.22
20	**CTL03 x G17NL544**	**75.92**	**77.58**	**1.66**	**2.91**	**1.75**	**6.82**	**32.65**	**31.06**	**0.91**	**19.05**	**8.30**
21	**CTL03 x G16NL860**	**80.51**	**81.22**	**0.71**	**2.54**	**1.40**	**12.36**	**32.09**	**25.63**	**0.83**	**20.95**	**8.26**
22	GS-PL07 x G16NL721	80.10	82.46	2.36	2.81	1.58	11.49	30.63	24.67	0.82	16.71	8.01
23	15AG163 x G16NL679	80.99	81.15	0.16	2.83	1.51	17.24	31.23	24.15	0.84	21.28	7.49
24	15AG143 x G16NL679	79.28	81.74	2.46	2.64	1.27	18.91	28.72	24.20	0.85	20.45	7.65
25	15AG143 x G17NL642	78.62	81.20	2.58	2.25	1.22	16.70	34.33	27.96	0.84	20.75	8.22
26	CTL03 x G16NL679	81.15	81.53	0.38	2.64	1.55	8.02	32.13	27.51	0.81	22.51	8.10
27	G15NL304 x G16NL679	85.35	85.89	0.54	2.04	1.21	6.48	23.37	20.62	0.82	19.51	5.85
28	15AG143 x G17NL544	76.67	78.33	1.66	2.13	1.22	6.55	26.06	24.30	0.89	21.29	7.44
29	**15AG162 x G16NL679**	**75.57**	**77.53**	**1.96**	**2.27**	**1.04**	**7.17**	**32.13**	**25.73**	**0.82**	**19.58**	**8.56**
30	15AG163 x G17NL544	79.49	80.73	1.24	2.41	1.43	12.09	29.15	25.06	0.81	21.28	8.16
	Mean of experimental hybrids	78.73	79.84	1.11	2.56	1.41	8.66	29.86	25.07	0.85	19.08	7.71
	CV (%)	3.12	2.82	74.86	9.75	14.94	41.23	10.85	10.65	3.54	9.49	13.48
	SD	2.46	2.25	0.83	0.25	0.21	3.57	3.24	2.67	0.03	1.81	1.04
	SE	0.45	0.41	0.15	0.03	0.04	0.65	0.59	0.49	0.01	0.33	0.19
	Commercial check hybrids											
31	SC 719	74.66	78.09	3.43	2.18	1.17	7.72	28.48	20.97	0.84	16.26	5.19
32	SC659	75.84	77.10	1.26	2.68	1.30	4.56	31.78	24.78	0.86	19.62	8.37
33	SC727	81.50	81.55	0.05	2.71	1.33	7.39	30.88	21.37	0.81	21.94	8.55
34	SC729	77.26	78.28	1.02	2.75	1.49	6.08	26.90	26.77	0.95	20.92	8.28
35	PAN7M81	77.91	78.06	0.15	2.26	1.10	6.55	32.82	30.98	0.86	18.14	8.21
36	PGS65	74.71	76.29	1.58	2.75	1.38	9.22	33.94	28.66	0.81	19.05	7.77
	Mean of check hybrids	76.98	78.23	1.25	2.56	1.29	6.92	30.80	25.59	0.86	19.32	7.73
	CV (%)	3.34	2.28	98.53	10.17	10.82	22.83	8.64	15.59	5.85	10.45	16.43
	SD	2.57	1.78	1.23	0.26	0.14	1.58	2.66	3.99	0.05	2.02	1.27
	SE	1.05	0.73	0.50	0.11	0.06	0.65	1.09	1.63	0.02	0.82	0.52
	Overall statistics											
	Grand mean	78.44	79.57	1.13	2.56	1.39	8.37	30.02	25.16	0.85	19.12	7.72
	Heritability	0.78	0.64	0.58	0.82	0.78	0.35	0.62	0.48	0.43	0.68	0.74
	LSD (5%)	1.08	1.12	0.55	0.21	0.19	2.94	1.86	2.81	0.02	1.19	0.69
	CV (%)	3.17	2.78	77.74	9.36	14.42	39.67	10.33	11.25	3.53	9.36	13.48
	SD	2.49	2.21	0.88	0.24	0.20	3.32	3.10	2.83	0.03	1.79	1.04
	SE	0.42	0.37	0.15	0.04	0.03	0.55	0.52	0.47	0.00	0.30	0.17
	R^2	0.62	0.91	0.58	0.94	0.58	0.60	0.55	0.86	0.84	0.80	0.94

AD=anthesis date (days to 50% pollen shed), SD=silking date (days to 50% mid-silking), ASI=anthesis-to-silking interval (days), PH=plant height (metres), EH=ear height measure from the ground to the node that bears the upper most ear (metres), TL=total lodging (percentage), NP=number of plants per 6m^2, EHP=number of ears harvested per 6m^2, EPP=number of ears per plant, MOI=moisture content at grain harvest (%), GY=grain yield (t/ha), LSD=least significant difference at 5% probability level, CV=coefficient of variation (percentage), SD=standard deviation, SE=standard error, R^2 = coefficient of determination.

Bold-faced entries denote the top 10 hybrids

4.3.3 Mean performance of hybrids in Zambia

The top 10 maize hybrids in Zambia, selected for their high grain yield are presented in Table 4.6. The lowest days to mid-pollen shedding were recorded for G15NL304 x G16NL679 (70.15 days), GS-PL07 x G17NL544 (70.18 days), 15AG162 x G17NL544 (71.71 days) and 15AG163 x G16NL679 (71.83 days). Check hybrid PAN7M81 (71.09 days), exhibited comparatively low days to mid-pollen shedding. Similarly, the shortest days to mid-silking were observed for G15NL304 x G16NL679 (71.67 days), 15AG162 x G17NL544 (71.75 days), CTL03 x G16NL721 (73.10 days), and GS-PL07 x G16NL679 (73.23 days) for the top selections, which were comparable to check hybrid SC719, with 71.07 days. The following hybrids had reduced anthesis to silking interval: 15AG162 x G17NL544 (0.04 days), GS-PL07 x G16NL679 (0.76 days), CTL03 x G16NL721 (0.94 days) and CTL03 x G16NL679 (1.00 days). Hybrids with short plant height were G15NL304 x G16NL679 (2.04 m), followed by GS-PL07 x G16NL679 (2.05 m), CTL03 x G17NL642 (2.49 m) and GS-PL07 x G17NL544 (2.50 m). Hybrids with reduced ear height included CTL03 x G17NL544 (1.39 m), CTL03 x G16NL721 (1.52 m) and GS-PL07 x G17NL544 (1.57 m). Top selections with total lodging tolerance included GS-PL07 x G16NL679 (6.00%), G15NL304 x G16NL679 (6.18%) and GS-PL07 x G17NL544 (7.73%). The highest number of plants per $6m^2$ was recorded for hybrid 15AG162 x G17NL544 (37.07), followed by G15NL304 x G16NL679 (34.99), GS-PL07 x G16NL679 (34.93) and CTL03 x G16NL860 (34.85). The following hybrids were selected for their highest number of ears harvested per $6m^2$: 15AG162 x G17NL544 (33.83), CTL03 x G17NL642 (33.26) and GS-PL07 x G17NL544 (32.39). The mean number of ears per plant was 1.00 for the top 10 selections. Low grain moisture at harvesting was recorded for 15AG163 x G16NL679 (13.24%), followed by CTL03 x G16NL860 (13.29%) and CTL03 x G17NL642 (13.46%), making them desirable selections. Grain yield varied from 7.75 t/ha (CTL03 x G17NL642) to 8.32 t/ha (15AG162 x G17NL544). The highest grain yield was recorded for 15AG162 x G17NL544 (8.32 t/ha), followed by CTL03 x G17NL544 (8.18 t/ha), GS-PL07 x G17NL544 (8.11 t/ha) and CTL03 x G16N721 (7.85 t/ha). The highest yielding of the tested checks was hybrid SC727 with 7.78 t/ha. Heritability estimates were moderate to high (42 to 82%) for most traits. Relatively low (<10%) coefficient of variations were computed for the majority of the traits, except for ASI, TL and GY. The coefficient of determination values were high (>60%) for all the assessed traits.

Table 4.6 Mean values for 11 agronomic traits of the experimental and commercial hybrids evaluated in Zambia

Entry	Single cross	AD	SD	ASI	PH	EH	TL	NP	EHP	EPP	MOI	GY
	Experimental hybrids											
1	GS-PL07 x G17NL642	68.33	69.34	1.01	2.87	1.34	19.73	32.11	30.97	0.96	11.95	6.37
2	15AG163 x G17NL642	72.79	73.56	0.77	2.49	1.79	9.05	34.97	28.00	0.95	13.43	6.47
3	GS-PL07 x G16NL860	71.11	72.60	1.49	2.56	1.57	18.51	35.39	35.46	0.89	14.07	7.54
4	**GS-PL07 x G16NL679**	**72.47**	**73.23**	**0.76**	**2.05**	**1.78**	**6.00**	**34.93**	**28.23**	**0.92**	**14.04**	**7.82**
5	**CTL03 x G17NL642**	**71.99**	**74.52**	**2.53**	**2.49**	**1.72**	**14.79**	**34.74**	**33.26**	**0.92**	**13.46**	**7.75**
6	15AG162 x G16NL721	72.08	73.29	1.21	2.41	1.63	6.20	32.22	29.74	0.92	15.65	7.42
7	15AG143 x G16NL721	70.59	71.39	0.80	2.69	1.49	29.37	31.74	29.28	0.92	15.42	7.46
8	G15NL304 x G17NL544	74.35	74.84	0.49	2.73	1.62	6.41	36.15	35.37	0.88	16.78	7.57
9	G15NL304 x G17NL642	71.89	72.16	0.27	2.76	1.53	7.01	37.07	34.03	0.92	14.22	7.48
10	**GS-PL07 x G17NL544**	**70.18**	**73.42**	**3.24**	**2.50**	**1.57**	**7.73**	**31.90**	**32.39**	**0.95**	**13.88**	**8.11**
11	15AG163 x G16NL860	71.08	74.15	3.07	2.69	1.62	3.86	35.56	28.00	0.89	13.49	7.67
12	G15NL304 x G16NL860	73.30	74.82	1.52	2.49	1.77	5.84	33.80	31.20	0.86	14.23	7.49
13	15AG143 x G16NL860	73.10	74.31	1.21	2.52	1.64	18.41	34.39	35.81	0.94	13.97	7.63
14	15AG163 x G16NL721	73.80	73.88	0.08	2.99	1.55	29.86	31.94	30.51	0.90	12.90	7.74
15	**15AG162 x G17NL544**	**71.71**	**71.75**	**0.04**	**2.58**	**1.75**	**11.46**	**37.07**	**33.88**	**0.91**	**13.70**	**8.32**
16	15AG162 x G17NL642	72.44	73.39	0.95	2.89	1.56	11.93	34.75	34.55	0.92	13.70	7.17
17	15AG162 x G16NL860	74.41	76.13	1.72	2.43	1.50	6.73	35.57	30.14	0.90	14.60	6.64
18	**CTL03 x G16NL721**	**72.16**	**73.10**	**0.94**	**2.72**	**1.52**	**26.82**	**33.15**	**30.12**	**0.91**	**15.40**	**7.85**
19	G15NL304 x G16NL721	72.86	75.04	2.18	2.62	1.68	9.17	33.89	34.43	0.92	16.85	7.65
20	**CTL03 x G17NL544**	**72.28**	**74.78**	**2.50**	**2.91**	**1.39**	**22.54**	**34.09**	**31.66**	**0.93**	**14.33**	**8.18**
21	**CTL03 x G16NL860**	**72.50**	**73.75**	**1.25**	**2.54**	**1.70**	**19.08**	**34.85**	**31.83**	**0.91**	**13.29**	**7.75**
22	GS-PL07 x G16NL721	70.58	71.82	1.24	2.81	1.48	3.74	34.07	33.07	0.92	13.57	7.42
23	**15AG163 x G16NL679**	**71.83**	**73.59**	**1.76**	**2.83**	**1.65**	**7.73**	**33.57**	**30.45**	**0.88**	**13.24**	**7.85**
24	15AG143 x G16NL679	71.24	71.80	0.56	2.64	1.47	12.61	35.58	31.12	0.89	14.67	6.80
25	15AG143 x G17NL642	72.26	72.70	0.44	2.25	1.80	7.46	32.49	31.74	0.92	13.59	6.71
26	**CTL03 x G16NL679**	**71.83**	**72.83**	**1.00**	**2.64**	**1.63**	**18.33**	**32.39**	**31.97**	**0.99**	**13.13**	**7.75**
27	**G15NL304 x G16NL679**	**70.15**	**71.67**	**1.52**	**2.04**	**1.76**	**6.18**	**34.99**	**29.10**	**0.90**	**13.65**	**7.85**
28	15AG143 x G17NL544	72.37	73.39	1.02	2.13	1.70	11.41	36.90	32.96	0.89	13.31	7.26
29	15AG162 x G16NL679	72.17	73.17	1.00	2.27	1.76	11.79	34.55	34.55	0.92	13.54	6.69
30	15AG163 x G17NL544	71.73	73.25	1.52	2.41	1.57	6.37	34.65	30.84	0.89	13.50	6.58
	Mean of experimental hybrids	71.99	73.26	1.27	2.56	1.62	12.54	34.32	31.82	0.91	14.05	7.43
	CV (%)	1.76	1.84	63.01	9.75	7.42	60.14	4.55	7.16	3.28	7.62	7.13
	SD	1.27	1.35	0.80	0.25	0.12	7.54	1.56	2.28	0.03	1.07	0.53
	SE	0.23	0.25	0.15	0.05	0.02	1.38	0.28	0.42	0.01	0.20	0.10
	Commercial check hybrids											
31	SC 719	72.04	71.07	-0.97	2.18	1.79	27.18	32.88	30.71	0.88	14.28	6.93
32	SC659	73.82	75.08	1.26	2.68	1.68	8.58	34.30	33.64	0.88	13.66	7.75
33	SC727	73.08	73.91	0.83	2.71	1.63	9.53	33.48	33.23	0.91	14.52	7.78
34	SC729	72.86	73.68	0.82	2.75	1.49	17.35	36.78	29.89	0.81	14.50	7.69
35	PAN7M81	71.09	72.38	1.29	2.26	1.64	17.38	32.66	31.88	0.98	15.42	5.48
36	PGS65	72.51	73.75	1.24	2.75	1.42	20.70	30.14	26.00	0.91	15.45	6.00
	Mean of check hybrids	72.57	73.31	0.74	2.56	1.61	16.79	33.37	30.89	0.89	14.64	6.94
	CV (%)	1.30	1.90	116.78	10.17	8.08	41.58	6.50	9.03	5.59	4.71	14.27
	SD	0.94	1.39	0.87	0.26	0.13	6.98	2.17	2.79	0.05	0.69	0.99
	SE	0.38	0.57	0.36	0.11	0.05	2.85	0.89	1.14	0.02	0.28	0.40
	Overall statistics											
	Grand mean	72.08	73.27	1.18	2.56	1.62	13.25	34.16	31.67	0.91	14.15	7.35
	Heritability	0.82	0.79	0.59	0.64	0.61	0.48	0.72	0.71	0.42	0.82	0.76
	LSD (5%)	1.12	1.21	0.65	0.20	0.09	3.21	1.42	2.15	0.01	0.89	1.06
	CV (%)	1.68	1.80	68.52	9.36	7.43	56.02	4.83	7.33	3.29	7.21	9.78
	SD	1.21	1.32	0.81	0.24	0.12	7.42	1.65	2.32	0.03	1.02	6.83
	SE	0.20	0.22	0.14	0.04	0.02	1.24	0.28	0.39	0.01	0.17	1.14
	R^2	0.64	0.84	0.62	0.86	0.72	0.68	0.66	0.90	0.82	0.84	0.88

AD=anthesis date (days to 50% pollen shed), SD=silking date (days to 50% mid-silking), ASI=anthesis-to-silking interval (days), PH=plant height (metres), EH=ear height measure from the ground to the node that bears the upper most ear (metres), TL=total lodging (percentage), NP=number of plants per $6m^2$, EHP=number of ears harvested per 6m2, EPP=number of ears per plant, MOI=moisture content at grain harvest (%), GY=grain yield (t/ha), LSD=least significant difference at 5% probability level, CV=coefficient of variation (percentage), SD=standard deviation, SE=standard error, R^2 = coefficient of determination.

Bold-faced entries denote the top 10 hybrids

4.3.4 Mean performance of test hybrids across sites

Table 4.7 presents the mean values of 30 experimental and six check hybrids evaluated for agronomic traits across seven locations in Zimbabwe and Zambia. The days to mid-pollen shedding for the hybrids varied from 72.02 days (15AG163 x G17NL642) to 78.02 days (G15NL304 x G16NL679) across locations. Early flowering hybrids, possessing relatively reduced days to mid-pollen shedding were 15AG163 x G17NL642 (72.02 days), GSPL07 x G17NL642 (72.81 days) and 15AG162 x G16NL679 (73.87 days), making them stable performing selections comparable to check hybrids SC719 (73.35 days) and PGS65 (73.61 days). Early and ideal days to mid-silking were expressed by the new hybrids 15AG163 x G17NL642 (with 73.73 days), followed by GSPL07 x G17NL642 (73.36 days). Check hybrids SC719 (74.58 days) and PGS 65 (75.02 days) also exhibited relatively low days to mid-silking. The following hybrids had reduced anthesis to silking interval across locations: 15AG143 x G16NL721 (0.77 days), CTL03 x G17NL642 (0.82 day), CTL03 x G17NL544 (0.85 days), GS-PL07 x G17NL544 (0.85 day), and G15NL304 x G17NL544 (0.87 day). The best hybrids with short plant height included G15NL304 x G16NL679 (2.58 m), followed by 15AG163 x G17NL642 (2.62 m) and 15AG162 x G16NL721 (2.72 m) in a desired direction. SC719 (with 2.64 m) and PAN7M81 (2.66 m) had comparable plant heights among the check hybrids. The shortest ear height was recorded for the following hybrids: 15AG163 x G17NL642 (1.26 m), SC719 (1.31 m), G15NL304 x G16NL679 (1.34 m), PAN7M81 (1.37 m) and 15AG143 x G16NL679 (1.37 m). Total plant lodging varied from 6.29% (G15NL304 x G17NL544) to 16.57% (15AG143 x G16NL679). The hybrid designated as G15NL304 x G17NL544 with a value of 6.29% was relatively tolerant to lodging, followed by G15NL304 x G16NL679 (6.33%), SC659 (6.57%), G15NL304 x G16NL721 (6.59%) and 15AG162 x G17NL544 (6.67%). The number of plants per 6m^2 varied between 29.86 (15AG163 x G17NL642) to 33.47 (CTL03 x G16NL860). Hybrids with the highest GEGVs of plants per 6 m^2 included CTL03 x G16NL860 (33.47), 15AG143 x G17NL642 (33.41), G15NL304 x G17NL544 (33.39), CTL03 x G17NL544 (33.37) and 15AG162 x G16NL679 (33.34).

The highest values for ears harvested per 6m^2 were computed for CTL03 x G17NL544 (31.36), GS-PL07 x G17NL544 (30.53), 15AG143 x G16NL860 (30.37) and 15AG162 x G16NL679 (30.14) in a desirable direction. The values were comparable to the check variety PAN7M81, with a value of 31.43. The mean number of ears per plant was 1.00 for all the test genotypes across locations. Hybrid GS-PL07 x G17NL642 (13.65%) had relatively the lowest grain moisture content at harvesting, followed by 15AG163 x G17NL642 (14.88%), GSPL07 x G16NL721 (15.14%), GSPL07 x G16NL860 (15.25%) and 15AG143 x G16NL860 (15.27%) which are desirable hybrids. Grain yield varied from 4.85 t/ha (G15NL304 x G16NL679) to 8.38 t/ha (CTL03 x G16NL721). The most stable and highest-yielding new hybrids were CTL03 x G16NL721 (8.38 t/ha), followed by CTL03 x G17NL544 (8.24 t/ha), GSPL07 x G17NL544 (8.23 t/ha) and 15AG162 x G17NL544 (8.10 t/ha). SC727 (8.17 t/ha) was the highest-performing check hybrid with a comparable yield to the top performers. The coefficient of variation was low (< 13.08%) for most of the assessed traits, except TL (26.40%). Moderate to high (50 to 96%) coefficient of determination values were observed for all the traits across the test locations.

Table 4.7 Mean performance of experimental and commercial maize hybrids for 11 agronomic traits across seven test locations in Zimbabwe and Zambia

Entry	Single cross	AD	SD	ASI	PH	EH	TL	NP	EHP	EPP	MOI	GY
	Experimental hybrids											
1	GS-PL07 x G17NL642	72.81	74.36	1.04	2.80	1.40	7.90	32.71	29.27	0.89	13.65	7.47
2	15AG163 x G17NL642	72.20	73.73	1.09	2.62	1.26	8.28	26.86	24.19	0.91	14.88	5.36
3	GS-PL07 x G16NL860	75.78	77.04	1.39	2.91	1.44	8.90	32.43	28.05	0.87	15.25	7.85
4	GS-PL07 x G16NL679	76.03	76.49	1.14	2.78	1.46	9.04	30.50	26.38	0.87	16.94	7.20
5	**CTL03 x G17NL642**	**75.33**	**75.78**	**0.82**	**2.91**	**1.54**	**8.61**	**32.64**	**28.74**	**0.89**	**16.87**	**8.06**
6	15AG162 x G16NL721	76.38	77.16	1.12	2.72	1.40	9.71	31.99	27.23	0.87	17.64	7.79
7	15AG143 x G16NL721	75.68	75.90	0.77	2.92	1.56	7.03	32.09	28.05	0.88	16.51	7.29
8	**G15NL304 x G17NL544**	**77.19**	**77.37**	**0.87**	**2.97**	**1.55**	**6.29**	**33.39**	**28.90**	**0.87**	**18.35**	**8.01**
9	G15NL304 x G17NL642	76.67	77.60	1.09	3.04	1.67	6.69	33.14	29.71	0.88	17.14	7.77
10	**GS-PL07 x G17NL544**	**75.01**	**75.39**	**0.85**	**2.94**	**1.61**	**7.41**	**32.62**	**30.53**	**0.94**	**15.58**	**8.23**
11	15AG163 x G16NL860	75.06	76.19	1.12	2.93	1.53	9.67	32.95	29.45	0.87	16.16	7.58
12	G15NL304 x G16NL860	75.67	75.84	0.90	2.86	1.51	8.53	31.66	27.80	0.86	16.21	7.33
13	15AG143 x G16NL860	74.66	75.53	1.14	2.86	1.43	7.75	32.49	30.37	0.90	15.27	7.91
14	**15AG163 x G16NL721**	**75.96**	**76.33**	**0.95**	**2.99**	**1.61**	**8.53**	**31.80**	**27.43**	**0.87**	**16.68**	**8.01**
15	**15AG162 x G17NL544**	**75.55**	**76.25**	**0.95**	**2.93**	**1.51**	**6.67**	**32.79**	**28.48**	**0.88**	**15.93**	**8.10**
16	15AG162 x G17NL642	75.70	76.14	1.00	2.97	1.59	7.28	31.60	28.19	0.89	16.17	7.73
17	15AG162 x G16NL860	76.44	77.09	1.00	2.82	1.41	10.70	30.85	26.84	0.87	15.78	5.88
18	**CTL03 x G16NL721**	**76.23**	**76.83**	**0.95**	**2.99**	**1.64**	**6.96**	**32.68**	**29.24**	**0.89**	**16.80**	**8.38**
19	**G15NL304 x G16NL721**	**75.10**	**76.63**	**1.14**	**2.93**	**1.48**	**6.59**	**32.14**	**29.50**	**0.90**	**18.53**	**7.94**
20	**CTL03 x G17NL544**	**74.93**	**75.35**	**0.85**	**2.88**	**1.57**	**7.90**	**33.37**	**31.36**	**0.92**	**16.69**	**8.24**
21	**CTL03 x G16NL860**	**76.86**	**77.13**	**0.95**	**2.97**	**1.55**	**13.05**	**33.47**	**28.73**	**0.87**	**17.12**	**8.00**
22	GS-PL07 x G16NL721	75.96	77.14	1.17	2.92	1.53	12.86	32.35	28.87	0.87	15.14	7.71
23	15AG163 x G16NL679	76.49	77.29	1.19	2.99	1.58	15.60	32.40	27.30	0.86	17.26	7.67
24	15AG143 x G16NL679	75.26	76.77	1.22	2.74	1.37	16.57	32.15	27.66	0.87	17.56	7.22
25	15AG143 x G17NL642	75.44	76.95	1.19	2.74	1.38	12.08	33.41	29.85	0.88	17.17	7.47
26	**CTL03 x G16NL679**	**76.68**	**76.99**	**1.19**	**2.94**	**1.59**	**8.78**	**32.26**	**29.74**	**0.90**	**17.82**	**7.92**
27	G15NL304 x G16NL679	78.02	78.51	0.95	2.58	1.34	6.33	29.18	24.86	0.86	16.58	4.85
28	15AG143 x G17NL544	74.52	75.86	1.17	2.78	1.46	8.98	31.48	28.63	0.89	17.30	7.35
29	15AG162 x G16NL679	73.87	75.35	1.27	2.74	1.40	8.52	33.34	30.14	0.87	16.56	7.63
30	15AG163 x G17NL544	75.61	76.99	1.24	2.86	1.50	9.23	31.90	27.95	0.85	17.39	7.37
	Mean of experimental hybrids	75.57	76.40	1.06	2.87	1.50	9.08	32.09	28.45	0.88	16.56	7.51
	CV (%)	1.57	1.27	14.19	3.84	6.69	28.63	4.24	5.55	2.27	6.46	10.65
	SD	1.19	0.97	0.15	0.11	0.10	2.60	1.36	1.58	0.02	1.07	0.80
	SE	0.22	0.18	0.03	0.02	0.02	0.47	0.25	0.29	0.00	0.20	0.15
	Commercial check hybrids											
31	SC 719	73.35	74.58	1.17	2.64	1.31	10.14	30.68	25.84	0.86	15.27	6.06
32	SC659	74.83	76.09	1.07	2.94	1.49	6.57	33.04	29.21	0.87	16.64	8.06
33	SC727	77.29	77.73	1.14	2.90	1.48	8.46	32.18	27.30	0.86	18.23	8.17
34	SC729	75.06	75.98	1.19	2.90	1.49	8.81	31.84	28.33	0.88	17.71	7.98
35	PAN7M81	74.50	75.22	1.07	2.66	1.37	10.00	32.74	31.43	0.92	16.78	6.85
36	PGS65	73.61	75.02	1.09	2.80	1.40	10.79	32.04	27.33	0.86	17.25	6.88
	Mean of check hybrids	74.77	75.77	1.12	2.81	1.42	9.13	32.09	28.24	0.88	16.98	7.33
	CV (%)	1.89	1.48	4.46	4.63	5.62	16.76	2.56	6.83	2.29	6.01	11.73
	SD	1.41	1.12	0.05	0.13	0.08	1.53	0.82	1.93	0.02	1.02	0.86
	SE	0.58	0.46	0.02	0.05	0.03	0.62	0.33	0.79	0.01	0.42	0.35
	Overall statistics											
	Grand mean	75.44	76.29	1.07	2.86	1.48	9.09	32.09	28.41	0.88	16.63	7.48
	LSD (5%)	0.75	0.63	1.21	2.65	4.59	0.89	1.91	0.80	2.61	3.23	0.26
	CV (%)	1.63	1.31	13.08	5.59	6.76	26.40	3.93	5.60	2.27	6.25	10.56
	SD	1.23	1.00	0.14	0.16	0.10	2.40	1.26	1.59	0.02	1.04	0.79
	SE	0.21	0.17	0.02	0.03	0.02	0.40	0.21	0.27	0.00	0.17	0.13
	R^2	0.50	0.96	0.56	0.92	0.50	0.56	0.67	0.91	0.90	0.82	0.96

AD=anthesis date (days to 50% pollen shed), SD=silking date (days to 50% mid-silking), ASI=anthesis-to-silking interval (days), PH=plant height (metres), EH=ear height measure from the ground to node that bears the upper most ear (metres), TL=total lodging (percentage), NP=number of plants per 6m2, EHP=number of ears harvested per 6m^2, EPP=number of ears per plant, MOI=moisture content at grain harvest (%), GY=grain yield (t/ha), LSD=least significance difference at 5% probability level, CV=coefficient of variation (percentage), SD=standard deviation, SE=standard error, R^2=coefficient of determination (percentage),

Bold-faced entries denote the top 10 performing hybrids across locations.

4.3.5 Genetic gains, heritability and variance components

Table 4.8 summarises the genetic gains, variance components and heritability estimates for the assessed traits of the 30 experimental hybrids and six commercial maize hybrids evaluated in Zimbabwe and Zambia. The genetic gain estimates for days to mid-pollen, days to mid-silking, anthesis-to-silking interval, plant height, ear height, and lodging tolerance were negative in a desirable direction. Negative gains recorded for days to mid-pollen and days to mid-silking suggested that the selected hybrids mature earlier than the commercial check hybrids. The mean days to mid-pollen for the selected hybrids (MS) was 73.22 days, lower than the mean of the population (MP) (75.44 days), mean of all commercial checks (MC) (74.77 days) and mean of the best commercial check (MBC) (73.35 days). These values correspond to negative and ideal gains of -2.94%, -2.07% and -0.18% over the MP, MC and MBC, respectively. The MS for the days to mid-silking was 74.78 days lower than the MP (76.30 days), MC (75.77 days) and MBC (74.58 days), translating to negative gains of -2.39%, -1.70% and -0.13% over MP, MC and MBC, respectively. The ASI values for selected hybrids were lower (0.91) compared to MP (1.07), MC (1.12) and MBC (1.07). Plant height exhibited negative gains of -7.69% below MP, -0.75% below MBC and -6.04% below MC, signifying that the selected hybrids where shorter than the commercial checks. Negative gains were recorded for ear height with values of -11.28% below MP and -10.14% below MC, indicating that the selected hybrids exhibited lower and ideal ear placement than most commercial hybrid checks.

The selected hybrids exhibited ideal lodging tolerance compared to the commercial varieties, corresponding to negative genetic gains of -27.50%, -27.82% and -22.10% below MP, MC and MBC, respectively. Significant gains were recorded for the number of plants per $6m^2$, ears harvested per $6m^2$ and ears per plant. The realised genetic gain for the number of plants per $6m^2$ was 3.66%, 0.05% and 3.95% over MP, MBC and MC, respectively. For the number of ears harvested per $6m^2$, the MS was 30.75, higher than the MC (28.24) and MP (28.41), corresponding to genetic gains of 8.88% and 8.24%, respectively. The number of ears per plant recorded positive genetic gains of 5.68%, 2.20% and 5.68% over MP, MBC and MC, respectively. The selected hybrids exhibited faster dry down with lower moisture content of 14.73% compared to MP (16.63%), MC (16.98%) and MBC (15.26%), resulting in negative and ideal gains of -11.43%, -13.25% and -3.47% against MP, MC and MBC, respectively. Positive genetic gains were noted for grain yield. The MS value was 8.29 t/ha, higher than MP (7.48 t/ha), MC (7.33 t/ha) and MBC (8.17 t/ha), respectively, with realized genetic gains of 10.83%, 13.09% and 1.47% above MP, MC and MBC. The higher and positive genetic gains found in this study indicated a marked genetic improvement, priming the newly developed hybrids for the marketplace.

Heritability estimates guide selection gains. In this study, the computed heritability values were classified into three groups: high heritability traits - >60%, 40 to 60% - moderate heritability, and <40% - low heritability. The majority of traits assessed exhibited high heritability. Days-to-mid-pollen (94%), days-to-mid silking (92%), grain moisture at harvesting (80%), and grain yield (78%) notably showed very high heritability. Anthesis-to-silking interval (50%) showed medium heritability, while lodging tolerance and ears per plant recorded low heritability (38%). High phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) values were recorded for most of the assessed traits. Lodging tolerance exhibited the highest PCV of 108.97% and GCV of 80.90%. Grain yield had a PCV value of 29.26% and a GCV of 26.70%.

Genetic advance as a percentage of the mean (GAM, %) is classified as low (<10%), medium (10–20%) and high (>20%). High heritability and moderate GAM were recorded for days to mid-pollen at 94% and 10.31%, in that order. Days-to mid-silking exhibited high heritability and low GAM at 92% and 9.80%, respectively, suggesting the expression of dominance or epistasis gene actions in the test hybrids. Anthesis-to-silking interval had moderate heritability and high GAM at 50% and 44.86%, in that order. High heritability and high GAM were recorded for plant height at 62% and 44.06% and ear height (63% and 45.95%), in that order, making them ideal traits with high selection response. Lodging tolerance had low heritability and high GAM at 38% and 49.95%, respectively. The following traits: number of plants per 6m^2 (67%, 22.65%) and ears harvested per 6m^2 (74%, 50.93%), recorded high heritability and high GAM, which was desirable. Ears per plant exhibited low heritability of 38% and moderate GAM of 15.91%. Grain moisture content at harvest had high heritability and high GAM at 80% and 32.41%, in that order. High heritability and GAM were calculated for grain yield at 78% and 51.34%, respectively, guaranteeing successful maize improvement through the selection of genomic-predicted inbred lines.

Table 4.8 Population and genetic parameters of 30 experimental maize hybrids and six commercial check hybrids evaluated across seven locations in Zimbabwe and Zambia.

Parameters	**AD**	**SD**	**ASI**	**PH**	**EH**	**TL**	**NP**	**EHP**	**EPP**	**MOI**	**GY**
Mean											
Population (MP)	75.44	76.30	1.07	2.86	1.48	9.09	32.18	28.41	0.88	16.63	7.48
Selected hybrids (MS)	73.22	74.48	0.91	2.64	1.33	6.59	33.36	30.75	0.93	14.73	8.29
All commercial checks (MC)	74.77	75.77	1.12	2.81	1.48	9.13	32.09	28.24	0.88	16.98	7.33
Best commercial check (MBC)	73.35	74.58	1.07	2.66	1.31	8.46	33.34	31.43	0.91	15.26	8.17
Realized genetic gain relative to											
MP	-2.94	-2.39	-14.95	-7.69	-11.28	-27.50	3.66	8.24	5.68	-11.43	10.83
MBC	-0.18	-0.13	-14.95	-0.75	1.53	-22.10	0.05	-2.16	2.20	-3.47	1.47
MC	-2.07	-1.70	-18.75	-6.04	-10.14	-27.82	3.95	8.88	5.68	-13.25	13.09
Variance components and heritability											
Genotypic variance	3.83	5.55	0.08	0.06	0.06	54.08	0.91	11.69	0.01	5.22	3.99
Phenotypic variance	20.49	6.30	0.28	0.13	0.13	98.11	3.39	19.52	0.01	7.00	4.79
Residual variance	16.66	0.75	1.29	0.01	0.01	44.04	2.48	7.83	0.01	1.78	0.80
Broad sense heritability (H^2)	0.94	0.92	0.50	0.62	0.63	0.38	0.67	0.74	0.38	0.80	0.78
Genotypic coefficient of variation (%)	2.59	3.07	26.43	0.09	0.17	80.90	3.39	12.03	11.36	13.74	26.70
Phenotypic coefficient of variation (%)	6.00	3.29	49.45	0.13	0.24	108.97	5.72	15.55	11.36	15.91	29.26
Genetic advance (GA)	7.78	7.48	0.48	1.26	0.68	4.45	7.29	14.47	0.14	5.39	3.84
Genetic advance as a per cent of mean (GAM) (%)	10.31	9.80	44.86	44.06	45.95	48.95	22.65	50.93	15.91	32.41	51.34

AD=anthesis date (days to 50% pollen shed), SD=silking date (days to 50% mid-silking), ASI=anthesis-to-silking interval (days), PH=plant height (metres), EH=ear height measure from the ground to the node that bears the uppermost ear (metres), TL=total lodging (percentage), NP=number of plants per $6m^2$, EHP=number of ears harvested per $6m^2$, EPP=number of ears per plant, MOI=moisture content at grain harvest (%), GY=grain yield (t/ha),

4.3.6 Correlations among grain yield and component traits

The summary of the Pearson correlation analysis among the assessed agronomic traits are presented in Table 4.9. Positive and significant (P≤0.05) correlations were calculated between days to mid-pollen and days-to-mid-silking (r = 0.99), anthesis to silking interval (r = 0.22), ear height (r = 0.39), total lodging (r = 0.28) and grain moisture at harvesting (r = 0.70). Days to mid-pollen exhibited significant (P≤0.05) and negative correlations with the number of plants per 6m^2 (r = -0.82), ears harvested per 6m^2 (r =-0.77) and ears per plant (r = -0.30). Days to mid-silking had positive and significant (P≤0.05) correlations with anthesis to silking interval (r = 0.37), ear height (r = 0.37), total lodging (r = 0.28) and grain moisture content at harvesting (r = 0.73). There were negative and significant (P≤0.05) correlations between days to mid-silking and the number of plants per 6m^2 (r = -0.84), ears harvested per 6m^2 (r = -0.80) and ears per plant (r = -0.34). Anthesis-to-silking interval was significantly and positively correlated to grain moisture at harvesting (r = 0.39) and showed negative and significant correlations with the number of plants per 6m^2 (r = -0.32), ears harvested per 6m^2 (r = -0.44) and ears per plant (r = -0.35). Plant height exhibited significant (P≤0.05) and positive correlations with ear height (r = 0.73), but negative and significant correlation with total lodging (r = -0.18).

Ear height was positively and significantly (P≤0.05) correlated with grain moisture at harvesting (r = 0.25) and showed a negative and significant correlation with the number of plants per 6m^2 (r = -0.30) and ears harvested per 6m^2 (r = -0.25). Total lodging was negatively and significantly correlated with the number of plants per 6m^2 (r = -0.22), ears harvested per 6m^2 (r = -0.26), ears per plant (r = -0.19) and grain moisture at harvesting (r = -0.18). The number of plants per 6m^2 showed a positive and significant correlation with ears harvested per 6m^2 (r = 0.83) and ears per plant (r = 0.17). There was a negative and significant correlation between number of plants and grain moisture content at harvesting (r =-0.68). Ears harvested per 6m^2 was positively and significantly correlated to ears per plant (r = 0.68), while negatively and significantly correlated to grain moisture at harvesting (r = -0.61). Ears per plant exhibited a negative and significant (P≤0.05) correlation with grain moisture at harvesting.

Grain yield was positively and significantly correlated (P<0.05) with plant height (r = 0.54), ear height (r = 0.38), number of plants per 6m^2 (r = 0.50), number of ears harvested per 6m^2 (r = 0.28), number of ears per plant (r = 0.34) and grain moisture content at harvesting (r = 0.21). The number of plants per 6m^2 (r =

0.50) exhibited the highest correlation with grain yield. Significant and negative correlations were observed between grain yield and anthesis to silking interval (r = -0.32), grain yield and total lodging (r = -0.36).

Table 4.9 Pearson correlation coefficients and significance tests comparing 11 agronomic traits of 36 maize hybrids evaluated across seven locations in Zimbabwe and Zambia.

Traits	AD	SD	ASI	PH	EH	TL	NP	EHP	EPP	MOI
AD	-									
SD	0.99***	-								
ASI	0.22**	0.37***	-							
PH	0.11ns	0.10ns	-0.03ns	-						
EH	0.39***	0.37***	-0.02ns	0.73***	-					
TL	0.28***	0.28***	0.10ns	-0.18*	-0.01ns	-				
NP	-0.82***	-0.84***	-0.32***	-0.03ns	-0.30***	-0.22**	-			
EHP	-0.77***	-0.80***	-0.44***	0.01ns	-0.25**	-0.26**	0.83***	-		
EPP	-0.30***	-0.34***	-0.35***	0.05ns	-0.05ns	-0.19*	0.17*	0.68***	-	
MOI	0.70***	0.73***	0.39***	0.04ns	0.26**	0.18*	-0.68***	-0.61***	-0.19*	-
GY	0.03ns	0.01ns	-0.32***	0.54***	0.38***	-0.36***	0.50***	0.28***	0.34***	0.21***

AD=anthesis date (days to 50% pollen shed), SD=silking date (days to 50% mid-silking), ASI=anthesis-to-silking interval (days), PH=plant height (metres), EH=ear height measure from the ground to the node that bears the uppermost ear (metres), TL=total lodging (percentage), NP=number of plants per $6m^2$, EHP=number of ears harvested per $6m^2$, EPP=number of ears per plant, MOI=moisture content at grain harvest (%), GY=grain yield (t/ha), *; **; *** significance at P < 0.05, 0.01 and 0.001, respectively, ns=not significant

4.4 Discussion

4.4.1 Performance of maze hybrids for yield and component traits

Genetic variability for yield and component traits among candidate inbred lines is crucial for developing high-performing maize hybrids. The present study appraised the performance of single cross hybrids for grain yield and component traits. The crosses were made from inbred lines selected with the best GEGVs. The derived single cross-hybrids were tested in seven locations in Zimbabwe and Zambia to guide breeding and production. The combined analysis of variance revealed the presence of genetic variability among the test hybrids for grain yield and component traits (Table 4.4). The genotype-by-location interaction and location effects were significant for all the traits evaluated (P<0.05), suggesting differential responses of the test hybrids across locations.

Top performing hybrids were identified based on contrasting agronomic traits such as reduced days to mid-pollen, days to mid-silking, anthesis to silking interval, plant height, and better ear height, tolerance to total lodging, a higher number of plants per $6m^2$, ears harvested per $6m^2$, ears per plant and reduced grain moisture content at harvest (Tables 4.5, 4.6 and 4.7). Genomic prediction has been used to select high-value inbred lines and top-performing hybrids based on genetic predictions of agronomic traits, such as plant and ear height, lodging tolerance, husk cover, ear length, ear diameter, number of rows per cob, number of kernels per cob, 1000 kernel weight and grain yield (Liu et al., 2018; Yang et al., 2020). Genomic prediction is a quick approach to developing and deploying improved maize cultivars with marker-preferred agronomic traits (Windhausen et al., 2012; Crossa et al., 2013; Technow et al., 2014; Kadam et al., 2016). The approach enables the selection of desirable genotypes that can perform highly under optimal and drought stress conditions without significant yield penalty (Windhausen et al., 2012; Crossa et al., 2014; Varshney et al., 2017; Hickey et al., 2019).

The present study identified hybrids with reduced days to mid-pollen and days to mid-silking, namely, 15AG162 x G16NL679, CTL103 x G17NL544, G15NL304 x G16NL679 and 15AG162 x G17NL544 (Tables 4.5 and 4.6). These hybrids combined high yield and earliness traits. The findings agree with Duvick et al. (2004), who reported positive gains from early maturing hybrids under drought stress conditions. Early maturing varieties are suitable to enhance maize productivity in SSA, where low and unpredictable rainfall is prevalent due to climate change. Reduced days to mid-pollen, days to mid-silking, anthesis to silking interval, and early maturity have been used to generate early maturing maize varieties, thereby enhancing productivity and production under drought-prone locations (Bolaños & Edmeades, 1996; Araus et al., 2012; Badu-Apraku et al. 2013).

The use of inbred parents with low GEGVs for plant and ear height resulted in the selection of superior hybrids with reduced plant and ear heights, namely 15AG162 x G16NL679 and CTL03 x G17NL642 in Zimbabwe and G15NL304 x G16NL679 and GS-PL07 x G16NL679 in Zambia. Reduced plant and ear heights are critical for lodging tolerance, especially under fertile soil conditions. These findings agree with Barten et al. (2022), who reported that short hybrids exhibit reduced lodging when exposed to windy conditions. Reduced plant height and lodging tolerance enable the use of inorganic fertilizers, leading to high nutrient use efficiency and higher yields, particularly under heavy rainfall and leaching conditions (Amado et al., 2017; Mueller & Vyn, 2018). Kosola et al. (2023) reported that short maize varieties had yields comparable to tall ones and showed an increased allocation of nitrogen and dry matter to the grain relative to tall hybrids. The findings are contrary to (Carvalho et al., 2017; Belay, 2018; Aman et al., 2020), who noted that grain yields have reportedly increased because of taller genotypes. The high yield is attributed to high dry matter accumulation and mobilization, which is attributed to many leaves per plant. High susceptibility to lodging negatively impacts maize yield gains. The study identified the following hybrids for their lodging tolerance: GS-PL07 x G17NL642, G15NL304 x G17NL544, GS-PL07 x G16NL679 and G15NL304 x G16NL679 (Tables 4.5 and 4.6). These hybrids were developed from crosses of lines with low GEGVs for lodging tolerance (Table 4.1).

Important agronomic traits such as the number of plants harvested per 6m^2, the number of ears harvested per 6m^2, and the number of ears per plant were positively and significantly correlated. These traits conferred a positive direct effect on grain yield (Rafiq et al., 2010; Adu et al., 2016). The traits had an impact on the selection of high-yielding experimental varieties. The following hybrids were identified for their high numbers of plants per 6m^2, ears harvested per 6m^2 and ears per plant: GS-PL07 x G17NL544, GS-PL07 x G17NL642, CTL03 x G17NL544, 15AG162 x G17NL544, G15NL304 x G16NL649 and CTL03 x G17NL642 (Tables 4.5 and 4.6). This lineage of the top hybrids followed the top-selected lines with high GEGVs for the traits (Table 4.1). The findings suggested that lines with high GEGVs confer top-performing hybrids. The present findings agree with Nastasić et al. (2010) and Carvalho et al. (2017), who reported that selection for a high number of ears per plot and productive ears per plant can significantly increase grain yield in maize.

Grain moisture content is crucial for the long-term storage of maize without depending on artificial drying. The new crosses from reduced GEGVs for grain moisture content at harvest resulted in the development of superior hybrids. This enabled the selection of best hybrids, namely GS-PL07 x G17NL642, CTL03 x G17NL544 and CTL03 x G16NL721 in Zimbabwe and CTL03 x G16NL860 and CTL03 x G17NL642 in Zambia. These hybrids combined high yield potential and fast dry-down rates. The results agree with Technow et al. (2014), who reported a prediction accuracy of 59% using GBLUP for grain moisture content at harvest. The findings suggest that genomic prediction of parental lines enables a shift in maize hybrid breeding by focusing on the performance of experimental hybrids. Slow-drying hybrids will increase production costs for farmers due to the extra energy required for artificial grain drying and pre-harvest losses such as cob rots, lodging and insect and bird damage (Nzuve et al., 2014).

High yield is a prerequisite for the farmers, breeders and seed producers. Lines with high GEGVs for yield from different heterotic groups are ideal candidates to develop superior hybrids. The new hybrids such as

CTL03 x G16NL721, 15AG162 x G16NL679, 15AG163 x G17NL544, CTL03 x G17NL544, GS-PL07 x G17NL544 and CTL03 x G16NL721 were high yielders in Zimbabwe and Zambia (Tables 4.5 and 4.6). Notably, hybrids CTL03 x G16NL721, CTL03 x G17NL544 and GS-PL07 x G17NL544 had higher yield stability and wide adaptation (Table 4.7). Massman et al. (2013) and Beyene et al. (2015) reported that genomic prediction has resulted in the speed breeding of inbred lines with high general and specific combining abilities, allowing rapid hybrid variety development. Li et al. (2020) used GS in Northwest China, selecting 44 top-performing hybrids, leading to a 6% increase in grain yield compared to the commercially successful hybrid variety (Zhengdan 958).

Reportedly, GS has been found effective in predicting hybrid performance in maize (Guo et al., 2019; Schrag et al., 2019; Li et al., 2020). The current study confirmed that newly developed hybrids performed better than the existing commercial varieties. The high GEGVs on yield and agronomic traits of the lines led to superior hybrid performance, indicating the potential of using GEGVs in developing highly productive hybrids. This helps to reduce the breeding cycle and attain genetic gains quickly.

4.4.2 Estimates of variance components, heritability and genetic gains

The success of any maize breeding program depends on adequate genetic variation, trait heritability, and selection efficiency. In this study, the observed differences between PCV and GCV values were small for most of the assessed traits. The results were consistent with Mahmood et al. (2004) and Maphumulo et al. (2015), who reported smaller differences between PCV and GCV values on agronomic traits in early-maturing maize hybrids. The differences between the two coefficients of variation demonstrate the significant influence of the environment on the expression of the assessed traits, with GCV values consistently lower than PCV values. High values of PCV and GCV were recorded for ASI, TL, EHP, EPP, MOI, and GY, indicating significant genetic variation in the maize gene pool.

Heritability refers to the percentage of phenotypic variance attributable to genetic variance. The study revealed high heritability for most of the assessed traits, indicating minimal environmental influence. Rafiq et al. (2010), Bello et al. (2012), and Ogunniyan et al. (2014) reported high heritability for yield component traits in maize. A high heritability value (99.95%) was reported in maize by Ojo et al. (2006) when evaluating characteristics associated with grain yield in maize inbred lines. Higher heritability values indicate that the observed variation in grain yield is due to genetic factors, probably with negligible confounding effects from the environment. This finding underscores the potential for effective genetic improvement through selection. Conversely, low heritability (38%) were found for total lodging tolerance and the number of ears per plant. The lower values suggest that the two traits may have been influenced by the environmental factors that masked genetic contributions during the selection process.

Broad-sense heritability encompasses additive, dominant, and epistatic genetic effects. Consequently, trait selection based on broad-sense heritability only may be ineffective due to unpredictable genetic effects and progress (Olakojo and Olaoye, 2011; Aminu and Izge, 2012; Azam et al., 2015). When choosing superior crop varieties, trait heritability should be considered with genetic advance (Alam et al., 2022). Most traits, such as plant height, ear height, number of plants per $6m^2$, number of ears harvested per $6m^2$, grain moisture at harvesting, and grain yield exhibited both high heritability and high genetic advance. These traits are governed by additive gene action and are well-suited for selection programs. Previous studies have reported similar findings (Akeel et al., 2008; Ogunniyan et al., 2015; Alam et al., 2022). The present study identified traits with high heritability and moderate genetic advance, suggesting the influence of dominance or epistasis genetic effect. Alam et al. (2022) reported high heritability with moderate genetic advance for days to mid-pollen and days to mid-silking when evaluating agronomic traits in maize hybrids. Days to mid-silking (9.80%) showed low genetic advance, but it was matched by the high heritability (92%). Ogunniyan et al. (2015) reported low genetic advance and high heritability for days to mid-pollen and days to mid-silking in yellow elite inbred lines.

High yield gains were recorded in the present study due to the favourable response of the test hybrids to multiple traits (Table 4.7). Under optimal growing conditions, genetic gains in plant and ear heights, lodging tolerance, number of ears per plant and number of ears harvested per plot contributed to increased grain yield (Badu-Apraku et al., 2014; Al-Naggar et al., 2016; Abdulmalik et al., 2017; Sun et al., 2017; Oyekunle et al., 2019). Improved crop management practices, such as optimum plant population, crop rotation, optimum sowing depth, correct planting time, weeding, insect pests and diseases control and fertilizer management bolstered maize yields globally (Qian et al., 2016; Liu et al., 2017).

The main factor affecting a new maize hybrid's commercial viability and competitiveness is its performance compared to existing commercial varieties. The study found high genetic gains of the newly developed hybrids over the means of the population, checks, and the best check variety (Table 4.8). The responses suggest that lines with high GEGVs can be crossed to create superior hybrids with better grain yield and agronomic characteristics. Cooper et al. (2014) developed 78 AQUAmax® single cross hybrids using genomic prediction adapted to USA drought stress conditions. The hybrids showed strong performance under optimal and drought conditions. Badu Apraku et al. (2019) reported yield gains of 16.9% and 12.6% in early maturing maize under striga-infested and optimal conditions, respectively. Their hybrids were bred using genomic selection for improved grain yield, striga and drought tolerance. Beyene et al. (2019) used genomic selection and identified the best hybrids with enhanced genetic gain. The authors indicated that the top 15% of selected hybrids showed a 21% increase in grain yield under well-watered conditions and a 52% increase under managed drought stress conditions compared to the average of the commercial checks.

4.4.3 The relationship between grain yield and component traits

Grain yield showed positive and significant correlations with plant height, ear height, number of plants per 6m^2, number of ears per plant, and grain moisture at harvesting (Table 4.9). This suggests that these traits can be used for direct selection during the development of high-yielding maize hybrids. Taller hybrids with high ear placement and prolificacy, high plant density and high grain moisture at harvesting resulted in higher grain yield potential (Shakoor et al., 2007; Nzuve et al., 2014; Carvalho et al., 2017; Aman et al., 2020). Pavan et al. (2011) and Marković et al. (2017) reported a strong association between grain yield and ear height in maize. Plant height and ear prolificacy were found to be positively and significantly correlated with grain yield (Ziyomo and Bernardo, 2013; Maphumulo et al., 2015; Adu et al., 2016; Carvalho et al., 2017). The positive correlation between plant height and ear height with grain yield suggests that taller plants with higher cob placement tend to have higher yields than shorter plants. Tall hybrids have large amounts of leaves, accumulating dry matter and leading to high yields (Nzuve et al., 2014). Furthermore, tall hybrids can efficiently mobilize photo assimilates to promote kernel expansion (Sujiprihati et al., 2003). Maphumulo et al. (2015) reported that breeding efforts prioritizing selection for short-statured plants may negatively impact grain yield potential. Conversely, increased plant height can be agronomically detrimental and limit grain yield through increased lodging (Pollak et al., 1991; Kaleem et al., 2013). Shorter hybrids produce fewer leaves, have a lower leaf area per plant, and pose fewer self-shading problems than tall cultivars. Shorter hybrids mature earlier than taller plants, making them suitable for drought-prone areas. Conversely, taller plants can be grown under favourable conditions to enhance maize production and productivity. Reportedly, increased plant density per unit area resulted in a leaf area index that maximizes solar radiation absorption, a crucial measure for optimizing maize grain yield (Zhang et al., 2021; Sun et al., 2023). The results of the study showed a positive relationship between grain yield and ear prolificacy. The expression of prolificacy (i.e., more than one fertile ear per plant) can lead to increased maize yields (Carvalho et al., 2017; Parco et al., 2020).

Grain yield was negatively and significantly correlated with anthesis-to-silking interval and total lodging tolerance. Shorter anthesis-to-silking intervals leads to fewer barren plants, allowing hybrids to withstand environmental stresses and maintain high planting densities and productivity (Bolaños & Edmeades, 1996; Duvick et al., 2004; Araus et al., 2012; Badu-Apraku et al., 2013). The negative correlation between grain yield and total lodging indicates that yield reduction is associated with total lodging. Lodged plants cannot transfer nutrients from the source to the sink (Nzuve et al., 2014). Therefore, selecting based on an ideal plant stand can effectively increase grain yield.

4.5 Conclusions

Genomic-assisted breeding techniques can improve crop yield and yield components by developing single cross-maize hybrids from inbred lines with the highest predicted GEGVs. This approach can enhance maize production and productivity under varied environmental conditions. A combined analysis of variance revealed significant variation among the test hybrids for the assessed traits. Significant yield gains were realized over the mean of the checks, population and best check variety. Moderate to high broad-sense heritability and genetic advance were recorded for most of the assessed traits, indicating the success of selection assisted by genomic predictions. The study identified three best single crosses combining high and stable yields and recommended for commercialization. Genomic prediction is a powerful tool for improving breeding efficiency by enhancing genetic gains and reducing breeding cycles and costs.

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CHAPTER FIVE

Combining Ability, Hybrid Prediction, and Genotype by Environment Interaction Analyses of Single-Cross Maize Hybrids Derived from Genomic Predicted Lines

Abstract

Analyses of the combining ability effects of elite parental lines and hybrid performance across the target production locations and seasons are crucial in maize breeding and commercial production. The objective of this study was to determine the combining ability effects of newly selected inbred lines and quantify the magnitude of heterosis and genotype by environmental interaction (GEI) effects of single cross hybrids to select and recommend contrasting elite lines and experimental hybrids. Six female and five male elite inbred lines selected through phenotyping and genomic predictions were crossed using a factorial mating design, and 30 single cross hybrids were derived and evaluated across seven locations in Zimbabwe and Zambia in the 2022/23 season. Significant ($P \leq 0.05$) general combining ability (GCA) effects of parental lines and specific combining ability (SCA) effects of crosses were recorded for grain yield and component traits. The following lines, CTL03, G17NL544, G16NL721, and GS-PL07, and crosses 15AG163 x G16NL679, G15NL304 x G17NL642, and 15AG162 x G16NL679, were good combiners for grain yield. The additive main effects and multiplicative interaction (AMMI) model revealed significant effects ($p \leq 0.001$) of genotype (G), environment (E), and GEI on grain yield, accounting for 38.95%, 50.58%, and 7.24% of the total variation, respectively. The test locations were clustered into two mega environments and recommended for hybrid selection and seed production. The genotype and genotype x environment (GGE) biplot analysis identified hybrids G15NL304 x G17NL544 and 15AG162 x G17NL544 as high-yielding and stable and identified for cultivation in Zimbabwe and Zambia and related agro-ecologies with enhanced productivity.

Key words: *combining ability analysis, genotype x environment interaction, hybrid breeding, mega-environments, yield stability*

5.1 Introduction

Maize (*Zea mays* L., 2n=2x=20) is a lucrative commodity crop cultivated in diverse agro ecological regions worldwide for food, feed and industrial purposes, including bioenergy production. The demand for maize is projected to increase by 30% in sub-Saharan Africa (SSA) by 2050 due to rising population growth and urbanization (Ekpa et al., 2018). The total maize production area in Africa covers approximately 42 million hectares, resulting in an estimated annual grain yield of 91 million tonnes (FAOSTAT, 2024). Maize productivity in Africa remains low (< 2 t/ha), affected by recurrent and emerging abiotic (e.g. drought and heat stress, flooding, waterlogging, erosion and poor soil health) and biotic (e.g. diseases and insect pests) stresses, socio-economic constraints, policy and lack of locally adapted, market-preferred and high yielding cultivars (Cairns et al., 2013). To meet the projected increase in food demand, maize production and productivity must increase by 2.4% per year to double global production by 2050 (Ray et al., 2013). Maize yields can be harnessed through hybrid cultivars to enhance livelihoods and economic opportunities along the value chains (Devi and Singh, 2011). Hybrid maize production, mainly single cross hybrids (SCHs), is the primary driver for widespread adoption and success of global seed systems. SCHs and the derived hybrid vigour enhance grain and biomass yields and improve adaptability to diverse environmental conditions caused by climate change (Birchler et al., 2006; Lippman and Zamir, 2007; Wang et al., 2011). Furthermore, the production and maintenance of SCHs is easier than three-way or four-way crosses.

Heterosis is expressed due to allelic combinations among genetically diverse and complementary inbred lines (Birchler et al., 2006; Lippman and Zamir, 2007). Determining heterosis among inbred lines is crucial for pipeline hybrid breeding and seed production in the target production locations and seasons. Combining ability analysis of elite inbred lines in hybrid combinations is essential for the identification of the best breeding parents to optimize hybrid development and genetic gain. Factorial mating designs are employed to determine the best combiners and elucidate the mode of gene action and variance components. Two types of combining ability effects are discerned in quantitative genetic analysis, viz., the general combining ability (GCA) effects of parents and the specific combining ability (SCA) effects of crosses (Lal et al., 2023). The GCA refers to the average performance of a line in hybrid combinations often conditioned by the additive genetic effects, while SCA denotes the difference in hybrid performance compared to the parental performance resulting from non-additive gene action (e.g. dominance, epistasis and their interactions) (Devi et al., 2011, Aminu et al., 2014; Lal et al., 2023). Combining ability analysis is critical in maize breeding to select desirable parental lines with the best GCA and experimental hybrids with the best SCA effects for the marketplace.

Several researchers have illustrated the impact of combining ability analysis in selecting maize genotypes for yield and component traits. Adewale et al. (2023) identified promising maize inbred lines exhibiting high combining ability effects that aided development of high-yielding and stress-tolerant hybrids. Similarly, Makinde et al. (2023) found lines with excellent GCA for grain yield, pro-vitamin A content, and *Striga* tolerance, enabling the development of improved hybrids. Guo et al. (2024) identified lines with a high combining ability for *Fusarium* ear rot resistance and detected heterosis, facilitating the development of resistant hybrids through strategic crossing and breeding programs. Lal et al. (2023) identified parental lines with high GCA effects for grain yield, reporting minimal SCA effects and moderate heterosis (10.5 to 43.8%) in hybrid development. Abakemal et al. (2016) selected high-yielding and adaptable quality protein maize lines with good combining ability suitable for tropical highlands. To establish specific and broad adaptations and recommend the best hybrid varieties, multi-environment trials are mandatory in breeding programs (Cooper et al., 1996; Yan et al., 2007; Devi and Singh, 2011). Also, multi-environment trials (METs) are essential for understanding genotype by environment interactions (GEI), which are crucial for identifying stable genotypes with consistent performance across a set of production environments (Scapim et al., 2000; Yan and Tinker, 2006; Gauch et al., 2008). Abiotic and biotic stresses and soil health contribute to the variable responses of genotypes, affecting maize productivity and yield stability (Yadav et al., 2016). GEI causes variations in genotype performance, ranking and selection (Robert & Denis, 1996; Šimić et al., 2003). Partitioning of GEI to discern genetic and environmental contributions is crucial to infer selection strategies and cultivar design (Wolde et al., 2018).

Various statistical methods are used to analyze GEI. The additive main effects and multiplicative interaction (AMMI) model (Gauch et al., 2008), genotype and genotype-by-environment interaction biplot analysis (GGE biplot) (Yan et al., 2000; Yan and Tinker, 2006), and stability and cultivar superiority indices (CSI) (Lin and Binns, 1988) are utilized to assess cultivar stability and performance. The AMMI model extends the analysis of variance (ANOVA) by incorporating principal component analysis to partition total GEI effects (Zobel et al., 1988; Gauch, 2013). GGE biplots help identify genotypes with broad and specific adaptations, using average environment coordination (AEC) to evaluate yield potential and stability (Kaya et al., 2006; Dehghani et al., 2009). The CSI assesses genotypes based on their productivity and stability across different environments, pinpointing the top-performing genotypes (Lin and Binns, 1988; Huehn, 1990; Scapim et al., 2000).

There is a dearth of information that used genomic predicted lines to assess combining ability effects on grain yield and component traits in diverse environmental conditions. To breed new and high-yielding maize cultivars, genomic prediction was pursued, and 30 candidate SCHs were developed from top-performing lines with high genomic estimated genetic values (GEGVs) based on grain yield and yield components (Gunundu et al., unpublished data). The combining ability, and GEI analyses of the new SCHs should be investigated to select the best parents and new hybrids with high and stable grain yield. This will enable the selection and recommendation of the best combiners and representative test environments for maize production and breeding. In light of the background, this study aimed to determine the combining ability effects of newly selected inbred lines and quantify the magnitude of heterosis and genotype by environmental interaction (GEI) effects of derived single cross hybrids to recommend best-performing lines and experimental hybrids.

5.2 Materials and methods

5.2.1 Plant materials

The germplasm used in this study is described in Chapter 4.

5.2.2 Development of single crosses

The crosses were developed as described in Chapter 4.

5.2.3 Study locations

The description of study locations is presented in Chapter 4.

5.2.4 Experimental design and management

The experiment was designed, established and managed as described in Chapter 4.

5.2.5 Phenotypic data collected

Phenotypic data collection is as described in Chapter 4.

5.2.6 Data analysis

5.2.6.1 Analysis of variance and mean separation

Data analysis was performed as described in Chapter 4.

5.2.7 Combining ability analysis

5.2.7.1 Analysis of variance

Analysis of variance (ANOVA) was computed to discern the significance effects of the GCA and SCA and their interaction with the test locations using the GenStat software 18th version (Goedhart & Thissen, 2016) based on the following model (Kearsey and Pooni, 1996).

$$Y_{ijk} = \mu + M_i + F_j + MF_{ij} + ME_{ie} + FE_{je} + MFE_{ije} + E_e + R(E)_{ki} + \varepsilon_{ijk}$$

Where; Y_{ijk} = observed trait value, μ = mean effect, M_i = effect of the i^{th} male, F_j = effect of the j^{th} female, MF_{ij} = effect of interaction between i^{th} male and j^{th} female, ME_{ie} = effect of interaction between i^{th} male and e^{th} environment, FE_{je} = effect of interaction between j^{th} female and e^{th} environment, MFE_{ije} = effect of interaction between i^{th} male, j^{th} female and e^{th} environment, E_e = effect of e^{th} location, R= effect of k^{th} replication within the environment, ε_{ijk} = experimental error. The hybrid checks were excluded from the factorial analysis.

5.2.7.2 Calculation of GCA and SCA effects

The hybrid variation was partitioned into male and female parent main effects, providing two independent estimates of GCA effects. The male x female interaction estimated the SCA effects (Hallauer and Miranda, 1988; Kearsey and Pooni, 1996). The GCA effects for the parents and SCA effects of the crosses were calculated according to Kearsey and Pooni (1996). Due to the unbalanced number of males and females, the standard errors (SE) for male and female GCA effects were calculated separately.

5.2.7.3 Calculation of GCA effects

$$GCAi = (di - (1/2)\ (d1 + d2))$$

where: GCAi is the general combining ability effect of parent i, di is the deviation of parent i from the overall mean and d1 and d2 are the deviations of parents 1 and 2 from the overall mean.

Standard error for GCA**:** $SE(GCA) = \surd\ (MSr / (2 \times \text{number of parents} \times \text{number of replications}))$

Where: MSr is the mean square for replicates, Number of parents is the number of parents in the mating design and Number of replications is the number of replications used in the experiment.

5.2.7.4 Calculation of SCA effects

$$SCA = (h - (1/2)\ (p1 + p2))$$

Where: SCA is the specific combining ability effect, h is the mean of the hybrid (F1) progeny and p1 and p2 are the means of parents 1 and 2, respectively.

Standard error for SCA: $SE(SCA) = \surd\ (MSr / (2r(b-1)))$

Where: SE(SCA) is the standard error of the specific combining ability effect, MSr is the mean square for replicates (or blocks), r is the number of replicates (or blocks), b is the number of parents (or lines) in the cross.

The significance levels of GCA and SCA effects were determined by the t-test, using standard errors of GCA and SCA effects, respectively. The predominance of additive versus non-additive variance was determined by comparing the ratio of components of GCA variance to SCA variance according to the model suggested by Baker (1978).

5.2.8 High-parent heterosis

The percent increase or decrease of F1 hybrids over the better parent for grain yield was calculated using GY means to estimate possible heterotic effects as follows (Sharma and Singh, 1978; Ahmed et al, 2005):

High-parent heterosis (HPH) (%) = [(F1 – HPV)/HPV)] x 100

where F1 is the mean performance of the cross and HPV is the mean value of the highest-performing parent.

5.2.9 Additive main effect and multiplicative interaction (AMMI) analysis

The AMMI analysis was performed using raw data from the seven environments using GenStat version 18 (Goedhart & Thissen, 2016). The model initially fits separate effects for genotypes and environments, then incorporates interaction effects by principal component analysis for G x E interaction. The AMMI was calculated based on the following model (Crossa, 1990):

$$\mathrm{Y}_{ij} = \mu + \mathrm{g}_i + \mathrm{e}_j + \sum_{k=1}^{t} \lambda_k \, \alpha_{ik} \gamma_{jk} + \epsilon_{ij}$$

Where; Y_{ij} is the yield of genotype i^{th} in the j^{th} environment, μ is the grand mean, g_i is the mean of the i^{th} genotype minus the grand mean, e_j is the mean of j^{th} environment minus the grand mean, λ_k is the square root of the eigenvalue of the principal component analysis (PCA) axis, α_{ik} and γ_{jk} are the principal component scores for PCAk of the i^{th} genotype and jth environment, respectively, and ϵ_{ij} is the residual error.

5.2.10 Cultivar superiority index

The stability of hybrids across environments was estimated using the cultivar superiority index in the Breeding Management System (BMS) (version 25 (BMSv25)) according to the model by Lin and Binns (1988):

$$\mathrm{Pi} = \sum_{j=1}^{\mathrm{n}} \left(\frac{\mathrm{X}_{ij} - \mathrm{M}_j}{2\mathrm{n}} \right)^2$$

Pi is the mean square between the cultivar's yield and maximum yield in each environment, X_{ij} is the yield of the i^{th} genotype in the j^{th} environment, M_j is the maximum yield in the j^{th} environment, n is the number of environments.

5.2.11 Genotype plus genotype-by-environment interaction (GGE) biplot analysis

The GGE (Yan and Tinker, 2006) plot was used to visually represent the multi-environmental data. "Ideal" and "non-ideal" genotypes and environments were identified using GGE biplots, according to Yan and Kang (2003). The GGE biplots show the first two principal components (PC1 and PC2) obtained by conducting single-value decomposition on the locational yield data (Yan et al., 2000). Environment-focused scaling was used for environmental comparisons, while genotype-focused scaling was used for genotypic comparisons. The GGE biplots were created using GenStat 18th version (Goedhart and Thissen, 2016) as follows:

$$\mathrm{Y}_{ij} - \mu_{\mathrm{i}} - \beta_j = \sum_{k=1}^{t} \lambda_k \, \alpha_{ik}\gamma_{jk} + \epsilon_{ij}$$

where Y_{ij} is the mean performance of genotype *i* in environment *j*, μ is the grand mean, β_j is the main effect of environment *j*, λ_k is singular value of the k^{th} PC; and α_{ik} and γ_{jk} are scores of the i^{th} genotype and j^{th} environment, respectively, for the k^{th} PC. The term $\lambda_k\alpha_{ik}\gamma_{jk}$ is derived from k^{th} PC of the singular value decomposition (SVD) of the matrix. The α_{ik} and y_{jk} for k = 1, 2, 3 … are referred to as "primary," "secondary," "tertiary," … effects of genotypes and environments, respectively; ε_{ij} is the residual associated with genotype *i* and environment *j*.

5.3 Results

5.3.1 Analysis of variance

Combined analysis of variance for grain yield and component traits across locations are presented in Table 5.1. There were significant genotypic differences for most of the recorded traits. Further, significant ($p \leq 0.05$) genotype by location interaction effects were recorded for all the assessed traits.

Table 5.1 Mean squares and significant tests for grain yield and component traits of 30 newly developed hybrids and six check hybrids, evaluated across representative locations in Zimbabwe and Zambia during 2022/23 summer season.

Source of variation	**df**	**AD**	**SD**	**ASI**	**df**	**PH**	**EH**	**TL**	**NP**
Locations (L)	5	2656.57***	2945.80***	157.87***	6	18.39***	0.44***	4757.21***	799.99***
L*Replication (Rep)	6	26.34	1.26	17.31	7	0.03	0.03	199.61*	8.87
L*Rep*block	60	42.63	6.43***	36.56	70	0.05***	0.06***	196.74***	6.92*
Genotype (G)	35	40.97**	12.60***	39.18**	35	0.13***	0.13***	196.22***	6.78**
L*G	175	34.57**	2.83***	36.34**	210	0.02***	0.03***	161.38***	5.49**
Residual	150	33.31	1.5	39.26	175	0.02	0.02	88.07	4.96
Total	431	66.08	41.32	88.82	503	0.25	0.04	198.57	15.12
Trial statistics									
LSD (5 %)		1.47	1.4	0.79		0.11	0.13	12.77	1.27
CV (%)		11.06	8.72	535.8		16.63	12.8	177.9	12.04
SE		0.39	0.32	0.31		0.02	0.01	0.63	0.17
Source of variation	**df**	**EHP**	**EPP**	**df**	**MOI**	**GY**			
L	4	14710.06***	7.83***	6	1150.44***	592.94***			
L*Rep	5	34.51	0.02	7	5.667	2.834			
L*Rep*block	50	46.03***	0.03***	70	8.53***	2.62**			
G	35	39.03***	0.02***	35	14.00***	9.57***			
L*G	140	21.05***	0.01***	210	5.34***	2.49***			
Residual	125	15.65	0.01	175	3.56	1.6			
Total	359	178.57	0.1	503	20.07	10.07			
Trial statistics									
LSD (5%)		3.28	0.15		1.93	1.07			
CV (%)		51.16	37.51		26.24	37.86			
SE		0.79	0.02		0.20	0.14			

*; **; *** significance at $P < 0.05$, 0.01 and 0.001, respectively, df=degrees of freedom, AD=anthesis date (days to 50% pollen shedding), SD=silking date (days to 50% mid-silking), ASI=anthesis-to-silking interval (days), PH=plant height (metres), EH=ear height measured from the ground to the node that bears the upper most ear (metres), TL=total lodging (percentage), NP=number of plants per $6m^2$, EHP=number of ears harvested per $6m^2$, EPP=number of ears per plant, MOI=moisture content at grain harvest (%), GY=grain yield (t/ha), LSD=least significance difference at 5% probability level, CV=coefficient of variation (percentage), SE=standard error.

5.3.2 Mean performance of hybrids

Table 5.2 presents the mean values of 30 experimental and six check hybrids evaluated for 11 agronomic traits across seven locations. The days to mid-pollen shedding ranged from 72.02 days (for 15AG163 x G17NL642) to 78.02 days (G15NL304 x G16NL679) across locations. Notably, hybrids 15AG163 x

G17NL642 (72.02 days), GSPL07 x G17NL642 (72.81 days), and 15AG162 x G16NL679 (73.87 days) exhibited early flowering comparable to check hybrids SC719 (73.35 days) and PGS65 (73.61 days). Similarly, for days to mid-silking, 15AG163 x G17NL642 (73.73 days) and GSPL07 x G17NL642 (73.36 days) displayed early and optimal timing. Check hybrids SC719 (74.58 days) and PGS 65 (75.02 days) also exhibited relatively low days to mid-silking. Hybrids such as 15AG143 x G16NL721 (0.77 days), CTL03 x G17NL642 (0.82 days), and GS-PL07 x G17NL544 (0.85 days) showed reduced anthesis to silking intervals across different locations. The shortest plant height was recorded for hybrid G15NL304 x G16NL679 (2.58 m), followed by 15AG163 x G17NL642 (2.62 m), and 15AG162 x G16NL721 (2.72 m), which are desirable for cultivation. SC719 (with 2.64 m) and PAN7M81 (2.66 m) had comparable plant heights among the check hybrids The hybrids with the lowest ear heights were 15AG163 x G17NL642 (1.26 m), G15NL304 x G16NL679 (1.34 m), and 15AG143 x G16NL679 (1.37 m), these were comparable to check hybrids SC719 and PAN7M81 with 1.31m and 1.37m respectively. Total plant lodging varied from 6.29% (G15NL304 x G17NL544) to 16.57% (15AG143 x G16NL679). The hybrid designated as G15NL304 x G17NL544 with a value of 6.29% was relatively tolerant to lodging, followed by G15NL304 x G16NL679 (6.33%), SC659 (6.57%), G15NL304 x G16NL721 (6.59%) and 15AG162 x G17NL544 (6.67%). The number of plants per 6m² ranged from 29.86 (for 15AG163 x G17NL642) to 33.47 (for CTL03 x G16NL860). The hybrids CTL03 x G16NL860 (33.47), 15AG143 x G17NL642 (33.41), G15NL304 x G17NL544 (33.39), CTL03 x G17NL544 (33.37), and 15AG162 x G16NL679 (33.34) showed the highest values of number of plants per 6m². Number of ears harvested per 6m² were highest for CTL03 x G17NL544 (31.36), GS-PL07 x G17NL544 (30.53), 15AG143 x G16NL860 (30.37), and 15AG162 x G16NL679 (30.14). The values were comparable to the check variety PAN7M81, with a value of 31.43. The mean number of ears per plant (1.00), was consistent across all genotypes and locations. Hybrids such as GS-PL07 x G17NL642 (13.65%) and 15AG163 x G17NL642 (14.88%) exhibited relatively lower grain moisture content at harvest, which is desirable. Grain yield ranged from 4.85 t/ha (for G15NL304 x G16NL679) to 8.38 t/ha (for CTL03 x G16NL721). Notably, CTL03 x G16NL721 (8.38 t/ha), CTL03 x G17NL544 (8.24 t/ha), GSPL07 x G17NL544 (8.23 t/ha), and 15AG162 x G17NL544 (8.10 t/ha) were the most stable and highest-yielding hybrids. SC727 (8.17 t/ha) was the highest-performing check hybrid with a comparable yield to the top performers. The coefficient of variation was generally low (< 15.00%) for most traits, except for total lodging (26.40%). Coefficient of determination values ranged from moderate to high (50% to 96%) across all traits and test locations.

Table 5.2 Mean values of 11 phenotypic traits among 30 single cross and six commercial check hybrids across seven locations in Zimbabwe and Zambia during the 2022/23 summer season.

Entry	Single cross	AD	SD	ASI	PH	EH	TL	NP	EHP	EPP	MOI	GY
	Experimental hybrids											
1	GS-PL07 x G17NL642	72.81	74.36	1.04	2.80	1.40	7.90	32.71	29.27	0.89	13.65	7.47
2	15AG163 x G17NL642	72.20	73.73	1.09	2.62	1.26	8.28	26.86	24.19	0.91	14.88	5.36
3	GS-PL07 x G16NL860	75.78	77.04	1.39	2.91	1.44	8.90	32.43	28.05	0.87	15.25	7.85
4	GS-PL07 x G16NL679	76.03	76.49	1.14	2.78	1.46	9.04	30.50	26.38	0.87	16.94	7.20
5	**CTL03 x G17NL642**	**75.33**	**75.78**	**0.82**	**2.91**	**1.54**	**8.61**	**32.64**	**28.74**	**0.89**	**16.87**	**8.06**
6	15AG162 x G16NL721	76.38	77.16	1.12	2.72	1.40	9.71	31.99	27.23	0.87	17.64	7.79
7	15AG143 x G16NL721	75.68	75.90	0.77	2.92	1.56	7.03	32.09	28.05	0.88	16.51	7.29
8	**G15NL304 x G17NL544**	**77.19**	**77.37**	**0.87**	**2.97**	**1.55**	**6.29**	**33.39**	**28.90**	**0.87**	**18.35**	**8.01**
9	G15NL304 x G17NL642	76.67	77.60	1.09	3.04	1.67	6.69	33.14	29.71	0.88	17.14	7.77
10	**GS-PL07 x G17NL544**	**75.01**	**75.39**	**0.85**	**2.94**	**1.61**	**7.41**	**32.62**	**30.53**	**0.94**	**15.58**	**8.23**
11	15AG163 x G16NL860	75.06	76.19	1.12	2.93	1.53	9.67	32.95	29.45	0.87	16.16	7.58
12	G15NL304 x G16NL860	75.67	75.84	0.90	2.86	1.51	8.53	31.66	27.80	0.86	16.21	7.33
13	15AG143 x G16NL860	74.66	75.53	1.14	2.86	1.43	7.75	32.49	30.37	0.90	15.27	7.91
14	**15AG163 x G16NL721**	**75.96**	**76.33**	**0.95**	**2.99**	**1.61**	**8.53**	**31.80**	**27.43**	**0.87**	**16.68**	**8.01**
15	**15AG162 x G17NL544**	**75.55**	**76.25**	**0.95**	**2.93**	**1.51**	**6.67**	**32.79**	**28.48**	**0.88**	**15.93**	**8.10**
16	15AG162 x G17NL642	75.70	76.14	1.00	2.97	1.59	7.28	31.60	28.19	0.89	16.17	7.73
17	15AG162 x G16NL860	76.44	77.09	1.00	2.82	1.41	10.70	30.85	26.84	0.87	15.78	5.88
18	**CTL03 x G16NL721**	**76.23**	**76.83**	**0.95**	**2.99**	**1.64**	**6.96**	**32.68**	**29.24**	**0.89**	**16.80**	**8.38**
19	**G15NL304 x G16NL721**	**75.10**	**76.63**	**1.14**	**2.93**	**1.48**	**6.59**	**32.14**	**29.50**	**0.90**	**18.53**	**7.94**
20	**CTL03 x G17NL544**	**74.93**	**75.35**	**0.85**	**2.88**	**1.57**	**7.90**	**33.37**	**31.36**	**0.92**	**16.69**	**8.24**
21	**CTL03 x G16NL860**	**76.86**	**77.13**	**0.95**	**2.97**	**1.55**	**13.05**	**33.47**	**28.73**	**0.87**	**17.12**	**8.00**
22	GS-PL07 x G16NL721	75.96	77.14	1.17	2.92	1.53	12.86	32.35	28.87	0.87	15.14	7.71
23	15AG163 x G16NL679	76.49	77.29	1.19	2.99	1.58	15.60	32.40	27.30	0.86	17.26	7.67
24	15AG143 x G16NL679	75.26	76.77	1.22	2.74	1.37	16.57	32.15	27.66	0.87	17.56	7.22
25	15AG143 x G17NL642	75.44	76.95	1.19	2.74	1.38	12.08	33.41	29.85	0.88	17.17	7.47
26	**CTL03 x G16NL679**	**76.68**	**76.99**	**1.19**	**2.94**	**1.59**	**8.78**	**32.26**	**29.74**	**0.90**	**17.82**	**7.92**
27	G15NL304 x G16NL679	78.02	78.51	0.95	2.58	1.34	6.33	29.18	24.86	0.86	16.58	4.85
28	15AG143 x G17NL544	74.52	75.86	1.17	2.78	1.46	8.98	31.48	28.63	0.89	17.30	7.35
29	15AG162 x G16NL679	73.87	75.35	1.27	2.74	1.40	8.52	33.34	30.14	0.87	16.56	7.63
30	15AG163 x G17NL544	75.61	76.99	1.24	2.86	1.50	9.23	31.90	27.95	0.85	17.39	7.37
	Mean of experimental hybrids	75.57	76.40	1.06	2.87	1.50	9.08	32.09	28.45	0.88	16.56	7.51
	CV (%)	1.57	1.27	14.19	3.84	6.69	28.63	4.24	5.55	2.27	6.46	10.65
	SD	1.19	0.97	0.15	0.11	0.10	2.60	1.36	1.58	0.02	1.07	0.80
	SE	0.22	0.18	0.03	0.02	0.02	0.47	0.25	0.29	0.00	0.20	0.15
	Commercial check hybrids											
31	SC 719	73.35	74.58	1.17	2.64	1.31	10.14	30.68	25.84	0.86	15.27	6.06
32	SC659	74.83	76.09	1.07	2.94	1.49	6.57	33.04	29.21	0.87	16.64	8.06
33	SC727	77.29	77.73	1.14	2.90	1.48	8.46	32.18	27.30	0.86	18.23	8.17
34	SC729	75.06	75.98	1.19	2.90	1.49	8.81	31.84	28.33	0.88	17.71	7.98
35	PAN7M81	74.50	75.22	1.07	2.66	1.37	10.00	32.74	31.43	0.92	16.78	6.85
36	PGS65	73.61	75.02	1.09	2.80	1.40	10.79	32.04	27.33	0.86	17.25	6.88
	Mean of check hybrids	74.77	75.77	1.12	2.81	1.42	9.13	32.09	28.24	0.88	16.98	7.33
	CV (%)	1.89	1.48	4.46	4.63	5.62	16.76	2.56	6.83	2.29	6.01	11.73
	SD	1.41	1.12	0.05	0.13	0.08	1.53	0.82	1.93	0.02	1.02	0.86
	SE	0.58	0.46	0.02	0.05	0.03	0.62	0.33	0.79	0.01	0.42	0.35
	Overall statistics											
	Grand mean	75.44	76.29	1.07	2.86	1.48	9.09	32.09	28.41	0.88	16.63	7.48
	LSD (5%)	0.75	0.63	1.21	2.65	4.59	0.89	1.91	0.80	2.61	3.23	0.26
	CV (%)	1.63	1.31	13.08	5.59	6.76	26.40	3.93	5.60	2.27	6.25	10.56
	SD	1.23	1.00	0.14	0.16	0.10	2.40	1.26	1.59	0.02	1.04	0.79
	SE	0.21	0.17	0.02	0.03	0.02	0.40	0.21	0.27	0.00	0.17	0.13
	R^2	0.50	0.96	0.56	0.92	0.50	0.56	0.67	0.91	0.90	0.82	0.96
	Number of tested locations	6	6	6	7	7	7	7	5	5	7	7

AD=anthesis date (days to 50% pollen shed), SD=silking date (days to 50% mid-silking), ASI=anthesis-to-silking interval (days), PH=plant height (metres), EH=ear height measure from the ground to node that bears the upper most ear (metres), TL=total lodging (percentage), NP=number of plants per 6m2, EHP=number of ears harvested per 6m^2, EPP=number of ears per plant, MOI=moisture content at grain harvest (%), GY=grain yield (t/ha), LSD=least significance difference at 5% probability level, CV=coefficient of variation (percentage), SD=standard deviation, SE=standard error, R^2=coefficient of determination (percentage),

Bold-faced entries denote the top 10 performing hybrids across locations.

5.3.3 Combining ability analyses

Table 5.3 shows the combining ability analyses among eleven parental lines and their crosses across locations in Zimbabwe and Zambia during the 2022/23 summer season. The mean squares due to GCA and SCA were significant ($P \leq 0.05$) for most of the traits, except SCA for EPP. The locations had a highly significant effect ($P \leq 0.001$) on all the assessed traits. Further, significant GCA and SCA × location interaction effects were computed for all the assessed traits. Estimates of variance components revealed the preponderance of SCA variance over GCA variance for most of the traits considered. The Baker's ratio varied from 0.02 (PH and EH) to 0.82 (MOI).

Table 5.3 Mean squares and significant tests for grain yield and component traits of single crosses derived from 11 parent factorial crosses, evaluated across representative locations in Zimbabwe and Zambia during the 2022/23 summer season.

Source of variation	**df**	**AD**	**SD**	**ASI**	**df**	**PH**	**EH**	**TL**	**NP**
Location (L)	5	2.47***	4181*98***	176.89***	6	13.70***	6.38***	2482.90***	980.39***
Replication/L	6	30.04***	37.38***	0.40***	7	0.14***	0.06***	182.20***	21.94***
GCA_{Male}	4	36.94***	33.39***	0.56*	4	0.02*	0.01*	21.10*	44.51**
$\text{GCA}_{\text{Female}}$	5	39.99*	44.82***	1.90*	5	0.07**	0.04*	47.70*	5.65*
$\text{SCA}_{\text{Male x Female}}$	20	16.53*	15.92*	1.82*	20	0.10*	0.10*	155.50*	17.98*
GCA_{Male} x L	20	2.47*	5.46*	1.31*	24	0.02*	0.03*	209.50**	14.64*
$\text{GCA}_{\text{Female}}$ x L	25	5.01*	6.22**	1.38*	30	0.03*	0.04*	124.6*	14.86**
$\text{SCA}_{\text{Male x Female}}$ x L	100	3.69**	4.23**	1.00*	120	0.03**	0.03**	180.10***	15.88**
Error	174	4.45	4.94	0.96	203	0.03	0.03	133.2	14.56
Variances									
GCA		0.30	0.16	0.004		0.002	0.002	1.43	0.22
SCA		0.88	0.67	0.06		0.009	0.006	4.07	1.44
Baker's ratio		0.34	0.24	0.07		0.22	0.33	0.35	0.15
Trial statistics									
LSD (5%)		5.67	6.60	1.53		0.36	0.26	9.80	4.00
CV (%)		9.15	10.35	117.70		18.29	24.83	138.70	15.89
SD		6.92	7.80	1.86		0.48	0.35	13.05	5.32
SE		0.37	0.42	0.10		0.02	0.02	0.64	0.26

Source of variation	**df**	**EHP**	**EPP**	**df**	**MOI**	**GY**
L	4	2008.74***	0.25***	6	1153.53***	319.45***
R/L	5	5.10***	0.00*	7	0.11***	2.95***
GCA_{M}	4	47.57*	0.02*	4	0.58*	2.61**
GCA_{F}	5	55.49**	0.03*	5	1.15*	1.29**
$\text{SCA}_{\text{M x F}}$	20	33.04*	0.01	20	3.95*	3.43**
GCA_{M} x L	16	23.61*	0.03*	24	2.19*	4.13*
GCA_{F} x L	20	26.21***	0.02*	30	2.41*	3.54*
$\text{SCA}_{\text{M x F}}$ x L	80	26.48*	0.02*	120	3.11*	3.33*
Error	145	25.25	0.02	203	3.22	3.24
Variances						
GCA		0.44	0.01		0.37	0.09
SCA		1.68	0.11		0.45	0.44
Baker's ratio		0.26	0.10		0.82	0.20
Trial statistics						
LSD (5%)		7.15	0.16		3.40	2.13
CV (%)		20.57	16.32		27.98	34.92
SD		7.12	0.16		4.43	2.80
SE		0.46	0.01		0.22	0.14

*; **; *** significance at $P < 0.05$, 0.01 and 0.001, respectively, df=degrees of freedom, AD=anthesis date (days to 50% pollen shed), SD=silking date (days to 50% mid-silking), ASI=anthesis-to-silking interval (days), PH=plant height (metres), EH=ear height measured from the ground to the node that bears the upper most ear (metres), TL=total lodging (percentage), NP=number of plants per 6m^2, EHP=number of ears harvested per 6m^2, EPP=number of ears per plant, MOI=moisture content at grain harvest (%), GY=grain yield (t/ha), GCA=general combining ability effect, SCA=specific combining ability effect, GCA_{M} x L=the interaction effect of general combining ability effects of male lines by location, GCA_{F} x L= the interaction effect of general combining ability effects of female lines by location, $\text{SCA}_{\text{M x F}}$ x L= the specific combining ability effects of males and females by location, LSD=least significance difference at 5% probability level, CV=coefficient of variation (percentage), SD= standard deviation, SE=standard error.

5.3.4 General combining ability effects

Table 5.4 presents the general combining ability effects of the 11 lines for grain yield and component traits across seven locations in Zimbabwe and Zambia. Lines G16NL642 (-0.88 and -0.64), 15AG163 (-0.51 and -0.29), GS-PL07 (-0.45 and -0.32) and 15AG143 (-0.46 and -0.20) exhibited negative GCA effects for days to mid-pollen and days to mid-silking. Negative GCA effects for these traits are desirable, and the selected lines can be used to breed early-maturity hybrids. For anthesis-to-silking interval, line CTL03 (-0.11) had negative GCA effects, while most of the lines recorded positive GCA effects for the trait. Negative GCA effects for plant and ear heights were observed among lines 15AG162 (-0.03 and -0.03), 15AG143 (-0.06 and -0.05), G16NL642 (-0.02 and -0.02) and G16NL679 (-0.07 and -0.04), indicating their equal desirability for conferring low plant and ear heights. Lines G15NL304 (-2.20), G17NL544 (-1.33), G16NL642 (-0.61) and 15AG162 (-0.51) recorded negative effects for total lodging, making them ideal selections with good lodging tolerance. Positive and significant ($P \leq 0.05$) GCA effects for number of plants per 6m^2 and ears harvested per 6m^2 were recorded for CTL03 (0.80 and 1.11), G17NL544 (0.50 and 0.86), 15AG143 (0.24 and 0.46) and G16NL860 (0.22 and 0.09) making them desirable selections. Desirable negative GCA effects were recorded for grain moisture at harvesting for the following lines: GS-PL07 (with -1.25), G16NL860 (-0.60) and G16NL642 (-0.58). Lines CTL03 (0.41), G17NL544 (0.37) and G16NL721 (0.34) exhibited positive and significant ($P \leq 0.05$) GCA effects for grain yield.

Table 5.4 General combining ability effects of 11 maize parental lines for grain yield and component traits across seven locations in Zimbabwe and Zambia during the 2022/23 summer season.

LINE	AD	SD	ASI	PH	EH	TL	NP	EHP	EPP	MOI	GY
CTL03	0.44*	0.02	-0.11	0.07	0.08	-0.02	0.80**	1.11**	0.01	0.50*	0.41*
GS-PL07	-0.45	-0.32*	0.06	0.00	-0.01	0.14	0.03	0.17*	0.01	-1.25	0.18
15AG162	0.02	0.00	0.01	-0.03	-0.03	-0.51	0.03	-0.27	-0.01	-0.15	-0.08
15AG163	-0.51	-0.29	0.06	0.01	0.00	1.18**	-0.91	-1.18	-0.01	-0.09	-0.33
G15NL304	0.96**	0.79**	-0.07	0.01	0.01	-2.20	-0.19	-0.29	-0.01	0.80**	-0.33
15AG143	-0.46	-0.20*	0.04	-0.06	-0.05	1.40**	0.24*	0.46*	0.00	0.20	-0.06
G17NL544	-0.10	-0.20	-0.07	0.03	0.04	-1.33	0.50*	0.86**	0.01	0.31*	0.37*
G16NL721	0.32*	0.27*	-0.04	0.04	0.04	-0.47	0.09	-0.06	0.00	0.32*	0.34*
G16NL642	-0.88	-0.64	-0.02	-0.02	-0.02	-0.61	-0.36	-0.12	0.01	-0.58	-0.20
G16NL860	0.18	0.07	0.03	0.02	-0.02	0.69	0.22*	0.09	-0.01	-0.60	-0.09
G16NL679	0.49*	0.50*	0.10*	-0.07	-0.04	1.73**	-0.45	-0.77	-0.01	0.56*	-0.43
SE	0.43	0.22	0.10	0.06	0.04	1.03	0.21	0.14	0.00	0.05	0.16

*; **; *** denote significance at $P < 0.05$, 0.01 and 0.001, respectively, AD=days to mid-pollen, SD=days to mid silking, ASI=anthesis to silking interval(days), PH=plant height (m), TL=total lodging (percentage), NP=number of plants per 6m^2, EHP=number of ears harvested per 6m^2, EPP=ears per plant, MOI=grain moisture at harvesting (percentage), GY=grain yield (t/ha), SE=standard error

5.3.5 Specific Combining Ability Effects

Specific combining ability effects for the 30 experimental single crosses for grain yield and component traits are presented in Table 5.5. Crosses 15AG162 x G16NL679 (-2.21 and -1.55), 15AG163 x G17NL642 (-1.99 and -1.74), 15NL304 x G16NL721 (-1.75 and -0.83), GS-PL07 x G17NL642 (-1.43 and -1.08) and G15NL304 x G16NL860 (-1.04 x -1.42) exhibited negative SCA effects for days to mid-pollen and days to mid-silking. Hence, the selected crosses are best combiners for earliness. The majority of the crosses had positive SCA effects for days to mid-pollen and days to mid-silking. Negative SCA effects for ASI were recorded for the following hybrids: 15AG143 x G16NL721 (-0.29), followed by GS-PL07 x G17NL544 (-0.20), G15NL304 x G16NL679 (-0.14), 15AG163 x G16NL721 (-0.13), G15NL304 x G16NL860 (-0.12) and CTL03 x G17NL642 (-0.11). Negative effects for plant and ear height were recorded for 15AG163 x G17NL642 (-0.24 and -0.21), G15NL304 x G16NL679 (-0.22 and -0.13) and 15AG162 x G16NL721 (-0.16 and -0.10), making them ideal short statured hybrids with low ear placement among the selected hybrids. The new hybrids 15AG143 x G16NL860 (-3.42), 15AG143 x G16NL721 (-2.98), G15NL304 x G16NL679 (-2.28), GS-PL07 x G16NL679 (-1.91) and 15AG162 x G16NL679 (-1.78) exhibited negative SCA effects for total lodging tolerance. Positive and significant SCA effects for number of plants per 6m^2 and ears harvested per 6m^2 were recorded for the following hybrids: 15AG162 x G16NL679 (1.68 and 2.73), 15AG163 x G16NL679 (1.55 and 2.09), G15NL304 x

G17NL642 (1.60 and 1.68), 15AG143 x G17NL642 (1.45 and 1.06) and 15AG163 x G16NL679 (1.67 and 0.80), which are desirable hybrids. Hybrids G15NL304 x G16NL679 (-1.34), GS-PL07 x G17NL642 (-1.08), 15AG163 x G17NL642 (-1.01), 15AG143 x G16NL860 (-0.89) and 15AG162 x G17NL544 (-0.79) exhibited negative effects for grain moisture at harvesting, indicating suitability of the crosses with fast dry down. For grain yield, positive and significant SCA effects were recorded for 15AG163 x G16NL679 (with 0.90), followed by G15NL304 x G17NL642 (0.79), 15AG162 x G16NL679 (0.63), 15AG143 x G16NL860 (0.55) and 15AG162 x G17NL642 (0.50).

Table 5.5 Specific combining ability effects for grain yield and component traits of 30 single cross maize hybrids derived from crosses between lines with highest GEGVs for grain yield and component traits across seven locations in Zimbabwe and Zambia.

Single cross	**AD**	**SD**	**ASI**	**PH**	**EH**	**TL**	**NP**	**EHP**	**EPP**	**MOI**	**GY**
15AG143 x G16NL679	-0.34	0.07	0.02	0.00	-0.03	4.36***	0.28	-0.48	0.00	0.24*	0.20
15AG143 x G16NL721	0.25	-0.57	-0.29	0.07	0.08	-2.98	-0.32	-0.80	0.00	-0.57	-0.50
15AG143 x G16NL860	-0.63	-0.74	0.02	0.03	0.01	-3.42	-0.05	1.37**	0.02	-0.89	0.55*
15AG143 x G17NL544	-0.49	-0.14	0.14	-0.05	-0.02	-0.17	-1.35	-1.14	0.00	0.23*	-0.47
15AG143 x G17NL642	1.21**	1.39**	0.11	-0.05	-0.04	2.21	1.45***	1.06**	-0.01	0.99**	0.22
15AG162 x G16NL679	-2.21	-1.55	0.10	-0.02	-0.02	-1.78	1.68***	2.73***	0.00	-0.41	0.63**
15AG162 x G16NL721	0.48*	0.50	0.09	-0.16	-0.10	1.60	-0.21	-0.88	0.00	0.91**	0.02
15AG162 x G16NL860	0.68*	0.62**	-0.09	-0.04	-0.03	1.44	-1.48	-1.43	0.00	-0.04	-1.46
15AG162 x G17NL544	0.06	0.05	-0.05	0.07	0.01	-0.57	0.17	-0.56	-0.01	-0.79	0.30
15AG162 x G17NL642	0.99**	0.38	-0.05	0.16	0.15*	-0.69	-0.15	0.14	0.01	0.34*	0.50*
15AG163 x G16NL679	0.94**	0.68*	-0.03	0.18	0.12*	3.61***	1.67***	0.80**	0.00	0.23*	0.90**
15AG163 x G16NL721	0.58*	-0.04	-0.13	0.07	0.07	-1.26	0.53*	0.23	0.00	-0.11	0.47*
15AG163 x G16NL860	-0.18	0.01	-0.02	0.03	0.05	-1.28	1.55***	2.09***	0.01	0.29*	0.47*
15AG163 x G17NL544	0.65*	1.08**	0.19	-0.04	-0.03	0.30	0.21	-0.17	-0.03	0.61*	-0.20
15AG163 x G17NL642	-1.99	-1.74	-0.01	-0.24	-0.21	-1.37	-3.96	-2.95	0.03	-1.01	-1.64
CTL03 x G16NL679	0.19	0.07	0.14	0.07	0.05	-2.01	-0.17	0.95**	0.02	0.20*	0.23
CTL03 x G16NL721	-0.09	0.15	0.04	0.01	0.02	-1.63	-0.29	-0.26	0.00	-0.58	-0.08
CTL03 x G16NL860	0.68**	0.64*	-0.03	0.01	-0.01	3.30***	0.37	-0.92	-1.76	0.66*	-0.03
CTL03 x G17NL544	-0.97	-0.87	-0.03	-0.08	-0.05	0.17	-0.02	0.94**	0.02	-0.68	-0.25
CTL03 x G17NL642	0.20	0.00	-0.11	-0.01	-0.02	0.16	0.12	-0.70	-0.01	0.39*	0.14
G15NL304 x G16NL679	1.00**	0.82*	-0.14	-0.22	-0.13	-2.28	-2.27	-2.53	0.00	-1.34	-1.90
G15NL304 x G16NL721	-1.75	-0.83	0.19	0.01	-0.07	0.17	0.15	1.41**	0.03	0.85**	0.42
G15NL304 x G16NL860	-1.04	-1.42	-0.12	-0.04	0.02	0.96	-0.46**	-0.45	-0.01	-0.55	0.24
G15NL304 x G17NL544	0.76*	0.38	-0.05	0.07	0.00	0.74	0.98	-0.11	-0.01	0.68*	0.46
G15NL304 x G17NL642	1.02**	1.05**	0.12	0.19*	0.18*	0.41	1.60***	1.68**	0.00	0.36*	0.79**
GS-PL07 x G16NL679	0.52*	-0.09	1.14***	-0.02	0.01	-1.91	-1.17	-1.47	-0.01	1.07**	-0.06
GS-PL07 x G16NL721	0.63*	0.79*	0.09	0.01	0.00	4.11***	0.14	0.31	-0.02	-0.49	-0.32
GS-PL07 x G16NL860	0.48*	0.89*	0.25	0.02	-0.03	-1.01	0.09	-0.66	-0.01	0.54*	0.24
GS-PL07 x G17NL544	-0.01	-0.50	-0.20	0.04	0.08	-0.48	-0.01	1.05**	0.04	-0.04	0.17
GS-PL07 x G17NL642	-1.43	-1.08	-0.06	-0.05	-0.07	-0.71	0.95*	0.77*	-0.01	-1.08	-0.02
SE	0.44	0.56	0.32	0.19	0.12	2.75	0.44	0.73	0.00	0.17	0.46

*; **; *** denote significance at $P < 0.05$, 0.01 and 0.001, respectively, AD=days to mid-pollen, SD=days to mid silking, ASI=anthesis to silking interval(days), PH=plant height (m), TL=total lodging (percentage), NP=number of plants per $6m^2$, EHP=number of ears harvested per $6m^2$, EPP=ears per plant, MOI=grain moisture at harvesting (percentage), GY=grain yield (t/ha), SE=standard error

5.3.6 Parent versus hybrid per se performances and heterosis for grain yield

Table 5.6 presents the high parent heterosis (HPH) of grain yield for the experimental hybrids. The HPH of the experimental hybrids for GY was relatively low due to the yield-reducing confounding effects of low-yielding sites at KRC and STAP. HPH ranged from 7.93% (15AG143 x G17NL544) to 24.33% (CTL03 x G16NL721), with a mean of 15.31%. The highest HPH (24.33%) was exhibited by the cross CTL03 x G16NL721, followed by CTL03 x G17NL544 (21.00%), GSPL07 x G17NL544 (20.85%) and CTL03 x G16NL860 (20.12%). The best-performing hybrids shared either CTL03 or G17NL544 as parents. The *per se* performance of the two parents was good. Both CTL03 and G17NL544 also recorded significant and positive GCA effects for GY (Table 5.4).

Table 5.6 Positive high-parent heterosis (%) for grain yield expressed by 27 selected single cross-maize hybrids tested at seven locations in Zimbabwe and Zambia.

Entry	**Single cross**	**GY**	**HP**	**HPH%**
1	GS-PL07 x G17NL642	7.47	6.72	11.16
2	GS-PL07 x G16NL860	7.85	6.66	17.87
3	GS-PL07 x G16NL679	7.20	6.65	8.27
4	CTL03 x G17NL642	8.06	6.72	19.94
5	15AG162 x G16NL721	7.79	6.74	15.58
6	15AG143 x G16NL721	7.29	6.74	8.16
7	G15NL304 x G17NL544	8.01	6.81	17.62
8	G15NL304 x G17NL642	7.77	6.72	15.63
9	GS-PL07 x G17NL544	8.23	6.81	20.85
10	15AG163 x G16NL860	7.58	6.66	13.81
11	G15NL304 x G16NL860	7.33	6.66	10.06
12	15AG143 x G16NL860	7.91	6.66	18.77
13	15AG163 x G16NL721	8.01	6.74	18.84
14	15AG162 x G17NL544	8.10	6.81	18.94
15	15AG162 x G17NL642	7.73	6.72	15.03
16	CTL03 x G16NL721	8.38	6.74	24.33
17	G15NL304 x G16NL721	7.94	6.74	17.80
18	CTL03 x G17NL544	8.24	6.81	21.00
19	CTL03 x G16NL860	8.00	6.66	20.12
20	GS-PL07 x G16NL721	7.71	6.74	14.39
21	15AG163 x G16NL679	7.67	6.65	15.34
22	15AG143 x G16NL679	7.22	6.65	8.57
23	15AG143 x G17NL642	7.47	6.72	11.16
24	CTL03 x G16NL679	7.92	6.65	19.10
25	15AG143 x G17NL544	7.35	6.81	7.93
26	15AG162 x G16NL679	7.63	6.65	14.74
27	15AG163 x G17NL544	7.37	6.81	8.22

GYD=grain yield (t/ha), HP=high parent, HPH%=high parent heterosis (percentage)

5.3.7 Additive main effect and multiplicative interaction (AMMI) analysis

AMMI analysis for grain yield across seven locations in Zimbabwe and Zambia is presented in Table 5.7. The environments (E), genotypes (G), and genotypes by environment interactions (GEI) were highly significant ($P \leq 0.001$) and accounted for 50.58%, 38.95%, and 7.24% of the total observed variation, respectively. The first interaction principal component axis (IPCA1) and the second interaction principal component axes (IPCA2) were highly significant ($p \leq 0.001$) and accounted for 34.92% and 21.63% of the total GEI variation, respectively.

Table 5.7 AMMI analysis of variance for grain yield of 36 maize hybrids tested across seven locations in Zimbabwe and Zambia.

Source of variation	**df**	**SS**	**MSE**	**Total variation explained (%)**	**G x E explained (%)**
Total	503	3811	7.58		
Treatments	251	3639	14.50***		
Genotypes (G)	35	764	21.83***	38.95	
Environments (E)	6	2218	369.61***	50.58	
Block	7	66	9.49***	21.98	
Interactions (GEI)	210	657	3.13***	7.24	
IPCA 1	40	603	15.08***		34.92
IPCA 2	38	27	0.71***		21.63
Residuals	132	27	0.2		
Error	245	106	0.43		

*** significance level at $P < 0.001$, df=degrees of freedom, SS=sum of squares, MSE=mean square error, G=genotype, E=environment, IPCA= interaction principal component axis.

5.3.8 Stability and cultivar superiority analyses

Table 5.8 presents cultivar superiority indices of the 30 experimental and six check hybrids tested at seven locations in Zimbabwe and Zambia. The cultivar superiority indices varied from 0.16 (CTL03 x G16NL721) to 16.00 (15AG163 x G17NL642). The top high-yielding and most stable experimental hybrids included CTL03 x G16NL721 (yield of 8.38 t/ha with an index of 0.16), followed by CTL03 x G17NL642 (8.06 t/ha and 0.66), GS-PL07 x G17NL544 (8.23 t/ha and 0.79), CTL03 x G17NL544 (8.24

t/ha and 0.95), and G15NL304 x G17NL544 (8.01 t/ha and 1.64), in that order. Among the check hybrids, SC727 (8.27 t/ha and 0.61) and SC659 (8.06 t/ha and 1.29) demonstrated comparable high yields and stability across different locations. Conversely, experimental hybrids 15AG163 x G17NL642 (16.00), 15AG162 x G16NL860 (13.94) and GS-PL07 x G16NL679 (10.74) exhibited the least stability across locations. Similarly, check hybrids SC719 (14.68), PAN7M81 (11.06) and PGS65 (10.45) showed less stability across different testing locations.

Table 5.8 Ranks of the 30 experimental and 6 commercial check hybrids based on cultivar superiority index.

Rank	**Entry**	**Single cross**	**Cultivar superiority index**
Experimental hybrids			
28	1	GS-PL07 x G17NL642	7.70
36	2	15AG163 x G17NL642	16.00
16	3	GS-PL07 x G16NL860	3.98
32	4	GS-PL07 x G16NL679	10.74
3	5	CTL03 x G17NL642	0.66
23	6	15AG162 x G16NL721	6.09
29	7	15AG143 x G16NL721	8.74
7	8	G15NL304 x G17NL544	1.64
19	9	G15NL304 x G17NL642	5.12
4	10	GS-PL07 x G17NL544	0.79
20	11	15AG163 x G16NL860	5.27
26	12	G15NL304 x G16NL860	7.07
14	13	15AG143 x G16NL860	3.60
12	14	15AG163 x G16NL721	3.47
10	15	15AG162 x G17NL544	3.20
18	16	15AG162 x G17NL642	4.45
34	17	15AG162 x G16NL860	13.94
1	18	CTL03 x G16NL721	0.16
13	19	G15NL304 x G16NL721	3.57
5	20	CTL03 x G17NL544	0.95
8	21	CTL03 x G16NL860	2.08
22	22	GS-PL07 x G16NL721	6.04
15	23	15AG163 x G16NL679	3.63
25	24	15AG143 x G16NL679	6.83
21	25	15AG143 x G17NL642	5.73
11	26	CTL03 x G16NL679	3.23
30	27	G15NL304 x G16NL679	9.53
24	28	15AG143 x G17NL544	6.77
29	29	15AG162 x G16NL679	4.41
27	30	15AG163 x G17NL544	7.34
Commercial checks			
35	31	SC 719	14.68
6	32	SC659	1.29
2	33	SC727	0.61
9	34	SC729	2.81
33	35	PAN7M81	11.06
31	36	PGS65	10.45

5.3.9 Genotype and genotype x environment (GGE) biplot analysis

5.3.9.1 Which-won-were and mega-environment delineation

Figure 5.1 presents the GGE biplot polygon view of "which hybrid won where" pattern and mega-environment delineation. The polygon view indicated the best grain yield performing genotype(s) for each environment and group of environments. This figure is derived from symmetrical scaling and illustrates the genotype that prevailed in each pattern, with equal emphasis on both the test genotypes and environments. About 97.74% of the total GGE variation was explained by the first principal component (PC1) and second principal component (PC2). The first PC explained 58.54% of the total GGE variation. The GGE-biplot was divided into nine sectors and the environments were clustered into two sectors (Figure 5.1). The polygon view of the GGE biplot subdivides the target environments into sub-regions (mega-environments) and is used for mega-environment identification. The polygon view classified the environment markers into two mega-environments, with Rattray Arnold research Station (RARS), Agricultural Research Trust (ART), Mpongwe Research Station (MPRS) and Lusaka West Research Station (LWRS) falling into one mega-environment designated mega environment 1 (ME-1). Mkushi Research Station (MKRS), Stapleford Research Centre (STAP) and Kadoma Research Centre (KRC) constituted the other mega environment designated mega environment 2 (ME-2). The best winners for each mega-environment were determined by the "which-won-where" graph, with these genotypes located at the vertices of the convex-hull of the polygon. Among the genotypes, entry 8 (G15NL304 x G17NL544) was identified as the most outstanding genotype in ME-1. In ME-2, entry 15 (15AG162 x G17NL544) was the vertex genotype out-performing other hybrids. Entries 1 (GS-PL07 x G17NL642), 2 (15AG163 x G17NL642), 31 (SC719), and 35 (PAN7M81) were vertex genotypes located furthest from markers of all environments, implying that they gave the poorest yields across all environments. All hybrids inside the polygons did not respond to the environmental differences.

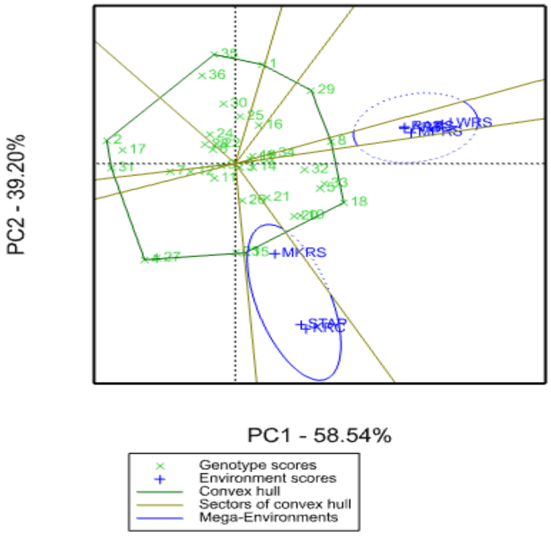

Figure 5.1 Polygon views of the GGE-biplot based on symmetrical scaling for “which-won-where” and mega-environment delineation. See codes of environments in Table 4.1 and genotypes in Table 5.2.

5.3.9.2 Ranking of genotypes relative to the ideal genotype

The rank of the genotypes relative to the ideal genotype based on the arrowed average environment coordinate (AEC) abscissa and various concentric circles of the GGE biplot is illustrated in Figure 5.2. The first two principal components explained 97.74% of the total variation in the GGE-biplot for comparison of genotypes with ideal genotypes (Figure 5.2). The results indicated that some genotypes were positioned closer to the center of the concentric circles, while others were relatively far from the origin of the concentric circles. Genotypes located at the centre of the concentric circles are regarded as the most ideal for high grain yield and stability. The hypothetical ideal genotype was used as a reference to the entries during evaluation. Among the new experimental hybrids, entries 5 (CTL03 x G17NL642), 10 (GS-PL07 x G17NL544), 14 (15AG163 x G16NL721), 18 (CTL03 x G16NL721), 20 (CTL03 x G17NL544) and 21 (CTL03 x G16NL860), were closest to the centre of the concentric circles in the GGE-biplot. As a result, they were placed close to the ideal reference genotype due to their high grain yield potential and stability compared to the other genotypes. Experimental entries 8 (G15NL304 x G17NL544), 16 (15AG162 x G17NL642) and 26 (CTL03 x G16NL679) were placed in the concentric circles for desirable genotypes and represented the next best genotypes. In contrast, experimental entries 2 (15AG163 x G17NL642), 17 (15AG162 x G16NL860) and 27 (G15NL304 x G16NL679) were positioned furthest away from the ideal concentric circle, indicating their unfavorable performance in terms of mean grain yield and stability across environments.

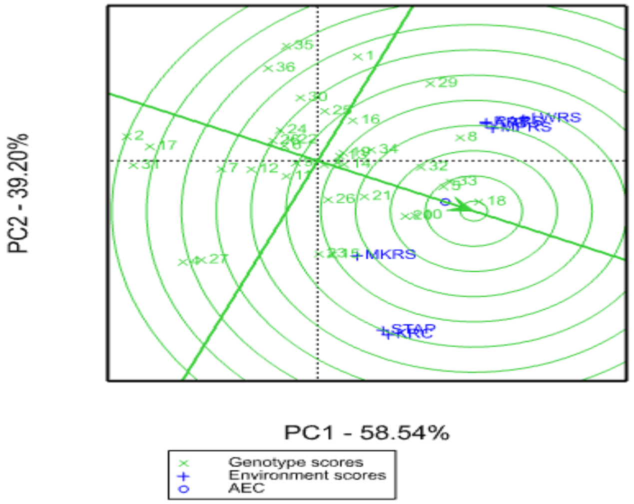

Figure 5.2 GGE- biplot comparing all genotypes to the ideal genotype, created using singular-value partitioning focused on both environment and genotype. See codes of environments in Table 4.1 and genotypes in Table 5.2.

5.3.9.3 GGE biplot analysis for the ideal environment

An ideal test environment should be both discriminating and representative (Yan and Kang, 2003). Figure 5.3 shows the GGE-biplot comparing the test locations with the ideal environment. The ideal environment was at the core of a series of concentric lines used for gauging the distance to the ideal environment from each surrounding environment. The biplot accounted for 97.74% of the total variation in relation to genotype and genotype-by-environment interaction. Based on the longest vector of environments and projection onto the AEC ordinate, the most representative environment with close proximity to the ideal environment was Mkushi Research Station (MKRS), while the least representative was KRC (Figure 5.3). All the environments were, however, considered favourable.

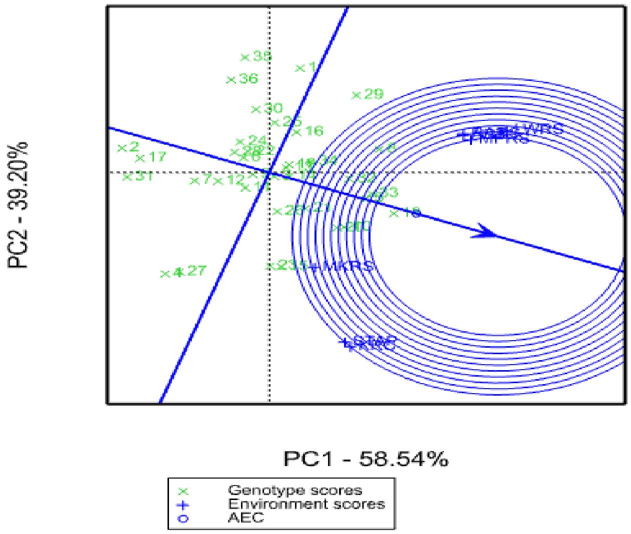

Figure 5.3 Biplot view of environment representativeness relative to the ideal testing environment. See codes of environments in Table 4.1 and genotypes in Table 5.2.

5.4 Discussion

5.4.1 Analysis of variance

Understanding combining ability, heterosis, and genotype by environment interaction is crucial for selecting genetically complementary inbred lines for the development of improved maize cultivars with market-preferred and essential traits. The combined analysis of variance revealed the presence of genetic variability among the test hybrids for grain yield and component traits (Table 5.1). The genotype-by-location interaction and location effects were significant for all the assessed traits ($P<0.05$), implying differential responses of the test hybrids across locations. This suggested that GEI influenced the performance of the test genotypes across locations. This necessitates further analysis to simplify the complexity of GEI using suitable statistical methods such as AMMI, GGE biplot models, and cultivar superiority index.

5.4.2 Mean performance of experimental maize hybrids for yield and component traits

Genomic prediction involves predicting the performance of hybrids based on the genetic makeup of parental lines for specific traits (Windhausen et al., 2012). This method has been used to select inbred lines and hybrids with desirable agronomic characteristics like plant and ear height, lodging tolerance, husk cover, ear size, kernel number, and ultimately grain yield (Liu et al., 2018; Yang et al., 2020). In the current study, genomic predicted inbred lines with high GEGV for yield and component traits were used to develop 30 single cross hybrids. The study identified the following top-performing hybrids with early flowering and maturity traits: 15AG163 x G17NL642, GSPL07 x G17NL642, 15AG162 x G16NL679, and GSPL07 x G17NL642 (Table 5.2). These hybrids exhibited reduced days to mid-pollen shedding and mid-silking. They could be beneficial for regions prone to drought stress as they have high yield potential and an early maturity period. Early-maturing varieties are treasured in areas with unpredictable rainfall patterns due to climate change. Traits related to early maturation have been essential in breeding programs aimed at improving maize productivity under drought-stress conditions (Bolaños & Edmeades, 1996; Araus et al., 2012; Badu-Apraku et al., 2013).

The selection of parents with reduced GEGVs for plant and ear heights resulted in the development of maize hybrids with shorter stature, such as G15NL304 x G16NL679, 15AG163 x G17NL642, 15AG162 x G16NL721, and 15AG143 x G16NL679. This reduction in plant height is significant for improving lodging tolerance, especially in nutrient-rich soil conditions. Shorter maize hybrids have demonstrated reduced susceptibility to lodging in windy conditions (Barten et al., 2022), offering improved stability. These hybrids exhibit enhanced nitrogen fertilizer efficiency, particularly in regions with high rainfall intensity (Mueller & Vyn, 2018). Furthermore, shorter varieties allocate more resources to grain production, boosting dry matter content, and thereby enhancing yield potential (Kosola et al., 2023). Hybrids with lodging tolerance were identified, including G15NL304 x G17NL544, G15NL304 x G16NL679, G15NL304 x G16NL721, and 15AG162 x G17NL544 (Table 5.2). These hybrids were developed from crosses involving lines with low GEGVs for lodging tolerance (Table 4.1).

The number of plants harvested per $6m^2$, the number of ears harvested per $6m^2$, and the number of ears per plant were positively correlated and directly impacted grain yield. Hybrids that had high numbers of plants per $6m^2$, ears harvested per $6m^2$, and ears per plant included CTL03 x G16NL860, 15AG143 x G17NL642, G15NL304 x G17NL544, CTL03 x G17NL544, 15AG162 x G16NL679, GS-PL07 x G17NL544, and 15AG143 x G16NL860 (Table 5.2). These hybrids were descended from top-selected lines with high GEGVs for these traits, highlighting the importance of choosing parental lines with desirable traits for hybrid development. The moisture content of the grain at harvest is crucial for maize storage, and hybrids with reduced GEGVs for grain moisture content were identified as superior, such as

GS-PL07 x G17NL642 and 15AG163 x G17NL642. These hybrids combined high yield potential with fast dry-down rates. These results support Technow et al.'s (2014) findings, which showed the reputability of genomic prediction for grain moisture content at harvest. Slow-drying hybrids can increase production costs and result in pre-harvest losses, underscoring the importance of selecting hybrids with favorable grain moisture content characteristics (Nzuve et al., 2014).

Higher grain yield is the ultimate goal of most plant-breeding programs, and lines with high GEGVs for grain yield from different heterotic groups are ideal candidates for developing superior hybrids. Hybrids such as CTL03 x G16NL721, CTL03 x G17NL544, and GS-PL07 x G17NL544 (Table 5.2), exhibited higher yield and stability, reflecting the effectiveness of genomic prediction techniques in maize breeding programs. The integration of genomic prediction techniques has accelerated the breeding process of inbred lines characterized by superior general and specific combining abilities within maize breeding programs (Massman et al., 2013; Beyene et al., 2015). Significant increases in yield have been observed using genomic selection, highlighting its potential for improving maize breeding programs (Li et al., 2020).

5.4.3 Combining ability effects of parental lines and hybrids

The additive (GCA) and non-additive (SCA) gene actions within a breeding population are important in determining which breeding procedure will efficiently improve the performance of the characteristics of interest. Selecting parental lines with good GCA for the traits of interest is crucial in hybrid breeding programs. These parents can potentially transfer favourable additive gene effects to their progenies, thereby improving the progenies with the target traits. Significantly higher GCA effects reveal the predominance of additive gene action, suggesting the potential for stable and predictable improvement through selection based on additive genetic variation. Significant interaction between GCA and SCA effects with environments were observed among the test inbred lines and their hybrids (Table 5.3), suggesting the importance of selecting parental lines and hybrids tailored to specific locations in that order. These results agree with Pswarayi and Vivek (2008), Musila et al. (2010), Wegary et al. (2011), and Estakhr and Heidari (2012) who reported that both GCA and SCA interact significantly with environments. The significance of GCA and SCA highlights the importance of genes with predominantly additive effects and those with dominance and epistatic effects, respectively (Baker, 1978). Thus, it becomes imperative to assess both the average performance of a line in hybrid combinations and the specific hybrid combinations. Effective selection or systematic hybridization among the best-combining inbred lines could be employed to enhance desired traits. Higher estimates of SCA variance over GCA variance were recorded for most of the assessed traits, indicating a predominant role of non-additive gene effects. This suggests that heterosis breeding could be highly rewarding in improving these traits.

5.4.4 General combining ability effects of parental lines

General combining ability assesses the genetic worth of parental lines and is critical in determining superior combiners in hybrid breeding. Integrating GCA into genomic prediction offers a powerful approach to optimise parental selection and accelerate gains in breeding programmes. Negative GCA effects for days to mid-pollen and days to mid-silking are preferred in breeding for early maturity. Early maturing cultivars are essential for cultivation in drought-prone areas. Predicted lines with negative effects for days to mid-pollen and days to mid-silking, namely G16NL642, 15AG163, GS-PL07, 15AG143 and G17NL544 (Table 5.4) are prime choices for hybrid breeding designed to achieve early flowering and maturity. These results are consistent with those of Matin et al. (2016), who reported negative GCA effects for maturity. According to Malik et al. (2004), earliness is generally associated with reduced days to mid-pollen and mid-silking. Early maturation is favoured for drought escape and reducing drought impacts under rainfed maize farming. Prioritization of early maturation has been emphasized in developing drought-tolerant maize varieties (Araus et al., 2012; Badu-Apraku et al., 2013). Across environments, the combining ability analyses indicated inbred lines with low GEGVs and negative GCA effects for anthesis to silking intervals, such as CTL03, G15NL304, G17NL544, G16NL721 and G16NL642 (Table 5.4). These lines can be used for drought-tolerance breeding. Parental lines with significantly negative GCA for ASI can be used in hybrid breeding for optimized pollen shed and silk reception, enhancing seed set and yield potential under drought conditions.

The following inbred lines, 15AG162, 15AG143, G16NL642, G16NL679 G15NL304 and G17NL544 exhibited significant negative GCA effects for plant height, ear height and lodging tolerance. These lines can be used to develop superior and short-stature maize varieties with good lodging tolerance. Rodrigues and Chaves (2002) and Aminu et al. (2014) reported related results for plant and ear height. Shorter plants with low ear height are associated with resistance to lodging, notably under fertile, waterlogging and windy conditions. Conversely, tall plants with high susceptibility to stem and root lodging negatively impact maize production and yield gains. Predicted lines CTL03, G17NL544, 15AG143 and G16NL860 (Table 5.4) exhibited positive and significant GCA effects for the number of plants per $6m^2$ and ears harvested per $6m^2$. These lines can be used in breeding to enhance maize yields. The results agree with Nastasić et al. (2010) and Carvalho et al. (2017), who reported yield gains in maize using parental lines with high numbers of plants and ears per plot.

The following lines: GS-PL07, G16NL860 and G16NL642, revealed significant and negative GCA effects for grain moisture content at harvesting. These lines could be used to develop fast-drying hybrids. Low grain moisture content is crucial for long-term storage of maize without depending on artificial drying. Lower grain moisture content can result in better grain quality, with reduced risks of moulding and mycotoxins (Nzuve et al., (2014). Predicted lines, such as CTL03, G17NL544, G16NL721 and GS-PL07, exhibited significant and positive GCA effects for grain yield (Table 5.4). This suggests that these parental lines could be used to develop high-yielding maize hybrids. These findings agree with Massman et al. (2013) and Beyene et al. (2015), who reported that genomic selection accelerates the breeding process, enabling the rapid development of inbred lines with good GCA and SCA, subsequently leading to the rapid creation of superior hybrids.

5.4.5 Specific combining ability effects

Specific combining ability is critical in predicting the outcome of crosses and identifying parents with the ability to produce hybrids with desirable traits. The present study identified hybrids with negative SCA effects for days to mid-pollen and days to mid-silking, namely, 15AG162 x G16NL679, 15AG163 x G17NL642, 15NL304 x G16NL721, GS-PL07 x G17NL642 and G15NL304 x G16NL860 (Table 5.5). These hybrids were ideal for early maturation. Early maturing hybrids can circumvent risks from weather extremes due to their shorter growth cycles. This is critical for drought escape under unpredictable rainfall conditions prevalent due to climate change effects. The findings agree with Duvick et al. (2004), who reported positive gains from early maturing hybrids under drought stress conditions. Hybrids 15AG163 x G17NL642, G15NL304 x G16NL679 and 15AG162 x G16NL721 manifested significant SCA effects in a negative direction for plant and ear height, indicating that the crosses had a good specific combination for shorter plant height and lower ear placement. Hybrids with short plant and ear height exhibit good lodging tolerance under fertile soil conditions. This necessitates easy harvesting and reduced grain loss.

Shorter plants with better lodging tolerance allow for easy inorganic fertilizers application, increasing nutrient use efficiency and yields (Amado et al., 2017; Mueller & Vyn, 2018).

Hybrids showing good lodging tolerance are ideal for enhanced yield gains. The study identified the following hybrids for their significant and negative SCA effects for total lodging tolerance: 15AG143 x G16NL860, 15AG143 x G16NL721, G15NL304 x G16NL679, GS-PL07 x G16NL679 and 15AG162 x G16NL679 (Table 5.5). These hybrids were developed from crosses of lines with negative GCA effects for lodging tolerance. Significant and positive SCA effects were observed among the following hybrids 15AG162 x G16NL679, 15AG163 x G16NL679, G15NL304 x G17NL642, 15AG143 x G17NL642 and 15AG163 x G16NL679 for essential agronomic traits such as plants harvested per $6m^2$, number of ears harvested per $6m^2$ and ears per plant. These characteristics had a direct beneficial impact on grain yield. The present findings agree with Nastasić et al. (2010) and Carvalho et al. (2017), who reported that selecting plants with many ears per plot and productive ears per plant can greatly boost maize yield.

Low grain moisture content enables long storage of hybrids with reduced risks of moulding. Additionally, low moisture content can also contribute to better germination rates and improved nutritional quality of the stored grain through the inhibition of biochemical processes that can degrade essential nutrients (Ziegler et al., 2021). The following new single crosses were selected with negative SCA effects for grain moisture content at harvesting: G15NL304 x G16NL679, GS-PL07 x G17NL642, 15AG163 x G17NL642, 15AG143 x G16NL860 and 15AG162 x G17NL544. These hybrids also exhibited high yield potential and fast dry-down rates. Slow-drying hybrids require artificial drying increasing production costs. They also get exposed to pre-harvest losses such as cob rots, lodging and insect and bird damage (Nzuve et al., 2014). High maize yields ensure stable food and feed supply and reduced risks of shortages. Achieving high maize yields is essential for promoting food security, reducing hunger, and fostering economic development in maize-growing regions. The study identified the following hybrids with positive SCA effects for grain yield: 15AG163 x G16NL679, G15NL304 x G17NL642, 15AG162 x G16NL679, 15AG143 x G16NL860 and 15AG162 x G17NL642 (Table 5.5). These hybrids were selected for their increased grain yield for direct production or further breeding. The identification of high yielding hybrids is a prerequisite of most maize breeding programmes. Massman et al. (2013) and Beyene et al. (2015) reported that development of superior hybrids with good specific combining ability for grain yield is made possible by using genomic predicted inbred lines with high GCA and SCA for grain yield.

The GCA effects of the selected parental lines were reflected in the SCA effects of the hybrids in some of the assessed traits. These results agree with Baker (1978), Paul and Duara, (1991), Malik et al. (2004) and Ahmed et al. (2017) who reported that the performance of single-cross progenies can be adequately predicted based on GCA effects. On the contrary, GCA effects of the parents were not reflected in the SCA effects of the crosses in some of the assessed traits, such as grain yield. This suggested that good general combining parents do not always show high SCA effects in their hybrid combinations. These findings agree with Ivy and Howlader (2000) who reported that good general combiners do not always show high SCA effects in their crosses. Hussain et al. (2003) and Deitos et al. (2006) also reported that parental lines with GCA for grain yield did not show high SCA effects in the hybrids.

5.4.6 High parent heterosis for grain yield

Exploiting heterosis in the maize gene pool is essential for enhancing grain yield in maize breeding programs. High parent heterosis is the superior performance of a hybrid compared to its best parent. The presence of high parent heterosis for GY in the present study indicated the superiority of the F1 hybrids to their parents. This superiority confirms the potential of these hybrids as valuable candidates for further breeding efforts aimed at developing superior hybrids, thereby enhancing genetic gains. The range of heterotic responses observed in this study (Table 5.6) was lower than those reported in studies by Betrán et al. (2003), Tollenaar et al. (2004), Wegary et al. (2013), Abera et al. (2016) and Demissew et al. (2016), who observed higher HPHs of over 100% for GY. The low levels of HPH suggest that though there is some improvement in grain yield in the hybrids compared to the better-performing parents, the degree of enhancement is relatively modest. This could be attributed to various factors, including the genetic similarity between the parents and environmental interactions (Hallauer and Miranda, 1988; Glover et al. 2005). While the modest yet positive HPH suggests that hybridization still holds the potential for improving grain yield, it also underscores the imperative need for further in-depth analysis into the underlying environmental dynamics governing yield performance. Understanding these factors is crucial for optimizing breeding strategies to unlock the full potential of heterosis and enhance maize productivity. Considering *per se* performance, SCA effect, and heterosis in hybrids is crucial for harnessing hybrid vigor and achieving significant improvements in maize. However, selecting based solely on one of these parameters may not be successful, since hybrids with strong *per se* performance may not always exhibit high SCA effects, and vice versa (Premlatha and Kalamani, 2010; Patil et al., 2012).

5.4.7 Additive main effects and multiplicative interaction

The AMMI model is crucial for analysing multi-environmental trials because it effectively reveals how different genotypes interact with various environments. It concisely summarises the observed patterns and relationships in these trials. AMMI analysis can extract a large portion of the genotype x environment interaction (GEI) and is more efficient in analysing GEI patterns in different crops, including maize (Zobel et al., 1988). The study carried out an AMMI analysis to determine the contribution of the environment, genotype, and GEI to the total observed variation. The genotypes, environments, and GEI were significant for grain yield, indicating a differential response of the test genotypes across the test environments (Table 5.7). The environment explained 50.58% of the total variance, followed by the genotype (38.95%) and GEI (7.24%). This indicated that the environments were highly variable and greatly influenced genotype performance. The results agree with Badu-Apraku et al. (2012), who reported that AMMI provided much insight into GEI. Significant genotype and genotype by environment effects were an indication of variability amongst the environments and single cross hybrids. These findings are consistent with several G x E studies carried out by other researchers in southern Africa (Setimela et al., 2005; Sibiya et al., 2011; 2013).

The influence of the seven locations on genotype performance could be attributed to differences in biotic, abiotic stresses and management practices. These findings agree with previous studies by Mafouasson et al. (2018), who reported environmental influence as the primary factor of variation in trials conducted across multiple environments. Test locations account for the largest variation in most multi-environmental trials compared to other factors (Badu-Apraku et al., 2012). Conversely, Mukherjee et al. (2013) reported that genotype major effects account for a larger percentage of the total variation. The cumulative variation (56.55%) confirms the considerable presence of GEI, which can be explained by the first two main components (IPCA1 and IPCA2). Mukherjee et al. (2013) reported that the IPCA scores of a genotype indicate the stability across environments. Previous studies have reported the existence of significant GEI and high environmental variation (Temesgen et al., 2015), highlighting the challenges resulting from GEI in crop breeding programs. AMMI analysis helped explain the GEIs in specific environments, resulting in the detection of suitable environments for each genotype group. Thus, the present study identified SCHs with specific positive adaptation to specific environments, which is a pattern of response that can be used in breeding suitable varieties for such environments.

5.4.8 Stability and cultivar superiority index (CSI) analysis

Identifying stable and high-grain yield hybrids with desirable traits in breeding programs is essential for maintaining production and productivity in maize (Prasanna et al., 2021). Lin and Binns (1988) state that superior cultivars have lower superiority indices. This was corroborated by Huehn (1990), who alluded that the stability rank gravitates towards zero when a hybrid attains maximum stability. The stability of grain yield in a cultivar indicates that its position of superiority compared to other cultivars remains consistent across a specific group of test locations (Scapim, 2000). Such varieties are the most productive and preferred in diverse agro-ecologies. The study identified experimental hybrids CTL03 x G16NL721, CTL03 x G17NL642, GS-PL07 x G17NL544, CTL03 x G17NL544 and G15NL304 x G17NL544 with high grain yield (Table 5.2) and low cultivar superiority indices (Table 5.8). This indicated the yield potential and wide adaptability of these hybrids across the test locations. The findings agree with Cooper et al. (2014) and Beyene et al. (2019), who reported high grain yield and stable maize hybrids across several testing locations. Farmers, breeders and seed producers prefer crop cultivars with high mean yields under different environments (Hallauer and Miranda, 1988). The experimental hybrids had exceptional performance over most of the commercial checks indicating the value of predicted lines with high GEGVs for yield and component traits. The lines offer potentially new sources of genes for higher grain yield potential and stability. Genetic gains are enhanced when newly constituted genotypes outperform productive commercial hybrids on the market. The top experimental hybrids are recommended for progression in yield trials towards possible commercialization. The top-yielding hybrids were more stable than the bottom performers, showing that breeding for wide adaptation might be a viable option. This significantly reduces the cost of breeding programmes through reduced multi-location evaluation and associated costs.

5.4.9 GGE biplot analysis of hybrids

The GGE biplots clustered the 36 maize hybrids into nine sectors and two mega-environments, respectively (Figure 5.1). These results indicate the diverse performance of genotypes across the test locations. In addition, the identified mega-environments could be used to understand the genotype by environment interactions and guide effective breeding strategies (Olanrewaju et al. 2021) Mega-environments represent a cluster of fairly similar regions consistently growing top cultivars (Nzuve et al., 2013). This implied diverse adaptation levels, where certain environments exhibit comparable discriminatory capacities and can substitute one another in multi-environment testing, reducing breeding costs. The GGE polygon view biplot identified experimental hybrids 8 (G15NL304 x G17NL544) and 15 (15AG162 x G17NL544) as top-performers in four and three environments, respectively. These hybrids could undergo additional testing with a view to release them in the environments where they have shown promising performance. The wide adaptability of these hybrids could also be exploited in future breeding initiatives, such as the prediction and development of three-way hybrids.

Mega environment 1, (Rattray Arnold Research Station (RARS), Agricultural Research Trust (ART), Mpongwe Research Station (MPRS) and Lusaka West Research Station (LWRS)) and mega environment 2 (Mkushi Research Station (MKRS), Stapleford Research Centre (SRC) and Kadoma Research Centre (KRC)) (Figure 5.1), exhibited similar top-performing varieties, suggesting a potential uniformity in their cropping systems and responses to both biotic and abiotic pressures. The identified mega environments could play a crucial role in minimizing the effect of environments, thereby reducing the costs associated with multi-location evaluation and enhancing the efficiency of hybrid maize development. The current findings agree with Samonte et al. (2005) and Mitrovia et al. (2012) who identified mega-environments based on the highest yielding genotypes shared across the grouped environments. In maize breeding efforts, choosing genotypes suited to niche environments is very crucial. Although genotypes may show wide adaptability, the heterogeneity of test locations necessitates their evaluation and deployment to specific environments. The significance of grouping test locations into multiple mega-environments, breeding and selecting maize genotypes suited for each mega-environment, despite varying cultivar rankings across locations is key (Gauch and Zobel, 1997). Using positive GEI in multi-environmental analysis involves segmenting the target sites into mega-environments and assigning distinct genotypes to each mega-environment (Yan and Tinker, 2005). The grouping of the seven test locations into two mega environments, suggests that cultivar information may be obtained using fewer test locations.

Genotypes exhibit varying performances in different environments owing to cross-over interactions, necessitating selections to be environment specific (Kamutando et al., 2013). The study observed instances of crossover rankings where varying genotypes achieved high grain yields at different test locations. The selection of two different hybrids in separate testing locations, as indicated by the GGE biplot "which won where" polygon view, suggests the presence of important cross-over effects and mega-environments, these results agree with Weikai et al. (2007) who reported different maize hybrids yielding highly at varying locations. The hybrids G15NL304 x G17NL544 and 15AG162 x G17NL544 were positioned at the vertex of the GGE-biplot polygon (Figure 5.1), indicating top performance in different mega-environments. The crossover rankings of these hybrids revealed the presence of significantly higher genotype by environment interaction. This finding supports the analysis of variance results showing that the interaction between environment and genotype accounted for 7.24% of the GGE variation.

GS has been used to predict grain yield in maize breeding, resulting in the development of superior cultivars (Liu et al. 2018; Yang et al. 2020). Experimental hybrids G15NL304 x G17NL544 and 15AG162 x G17NL544 developed from lines with predicted high GEGVs for yield and component traits out yielded the commercial checks in Zimbabwe and Zambia, respectively. This suggested that contrasting lines with high GEGVs can be used to develop superior hybrids for specific maize production environments in the two countries. Hybrids GS-PL07 x G17NL642, 15AG163 x G17NL642, and SC719 and PAN7M81(checks) were vertex genotypes located furthest from markers of all environments (Figure 5.1). This suggest that they gave the lowest yields across all environments. The findings agree with Nyombayire et al. (2018), who reported that maize genotypes did not exhibit any yield advantage in any of the identified mega-environments.

5.4.10 Genotype stability

Continuous breeding efforts aimed at releasing, deploying and seed marketing of stable and high yielding varieties are the major objectives of any seed company. High and stable performance are critical for the success and acceptance of new hybrids in the market. Ideal hybrids have positive extremes of high mean yield and remarkable stability across environments (Yan et al. 2000). GGE-biplot analysis effectively selects high yielding and stable genotypes in multi-environmental analyses of contrasting genotypes (Balestre et al., 2009). Genotypes closest to the hypothetical genotype represent superior genotypes, combining high grain yield and outstanding yield stability (Yan and Tinker, 2005; Yan et al., 2007). The ideal hybrid is represented by the arrowhead at the centre of concentric circles and has the longest vector on the AEC abscissa and its projection on the AEC ordinate is zero (Yan and Tinker, 2006). The distance from the arrowhead to the concentric rings determines genotypes that are closer to being ideal (Yan, 2001).

The study identified the following experimental hybrids: 5 (CTL03 x G17NL642), 10 (GS-PL07 x G17NL544), 14 (15AG163 x G16NL721), 18 (CTL03 x G16NL721), 20 (CTL03 x G17NL544) and 21 (CTL03 x G16NL860) as being closer to the ideal hybrid, combining high grain yield and good yield stability across the seven testing locations (Figure 5.2). The performance of the hybrids qualified them as potential candidates for commercialization and production in Zimbabwe and Zambia. Similarly, experimental hybrids 8 (G15NL304 x G17NL544), 16 (15AG162 x G17NL642) and 26 (CTL03 x G16NL679) placed in the concentric circles identifying them as second-best genotypes conferring high grain yield and stability (Figure 5.2). Successful hybrids in SSA have been found to combine high yield and broad adaptation (Windhausen et al., 2012). Identifying superior hybrids outcompeting commercial checks is crucial for the attainment of genetic gains. These findings agree with Sserumaga et al. (2018), who reported newly constituted hybrids outperforming check hybrids under drought stress and optimum conditions. Tonk et al. (2011) and Wolde et al. (2018) reported new experimental hybrids with high yields and good stability compared to check commercial hybrids.

The performance of the selected experimental hybrids over commercial checks was a result of superior lines used in the constitution of the single crosses. The crosses were made from contrasting inbred lines selected with the best GEGVs for yield and component traits (Table 4.1). This indicates the significance of genomic selection in selecting high-value inbred lines and top-performing hybrids with good yield and stability, based on genetic predictions of agronomic traits (Liu et al., 2018; Yang et al., 2020). Genomic prediction is a quick approach to developing and deploying improved maize cultivars with wide adaptability and market-preferred agronomic traits (Windhausen et al., 2012; Crossa et al., 2013; Technow et al., 2014; Kadam et al., 2016). The approach enables the selection of desirable genotypes with high performance under optimal and drought stress conditions without significant yield penalty (Windhausen et al., 2012; Crossa et al., 2014; Varshney et al., 2017; Hickey et al., 2019). The predicted lines with high GEGVs in the hybrid combinations should be explored further for their value in developing high yielding and stable maize hybrids preferred in SSA. Experimental hybrids 2 (15AG163 x G17NL642), 17 (15AG162 x G16NL860) and 27 (G15NL304 x G16NL679) were positioned furthest away from the ideal concentric circle, indicating their unfavourable yield performance across environments. These genotypes were not selected because of their static stability. The results agree with Kandus et al. (2010) who reported consistently low yielding hybrids in contrasting test locations.

5.4.11 Ideal environment

Genotype by environment analysis is the quest for understanding the effect of the environment on the performance of genotypes. The growing environment plays a significant role in the performance and selection of hybrids. All genotypes are dependent on the environment in which they are grown for their performance. The environment in which the genotype will perform best is termed the 'ideal environment'. The 'ideal environment' concept holds immense importance to plant breeders and producers. Environmental conditions regulate gene expression, resulting in genotype × environment interaction (GEI). Comprehending GEI helps in pinpointing genotypes showing consistent yield performance across diverse environments (Chapman, 2008). An ideal environment has a high capacity for genotype discrimination and selection of generally adapted genotypes (Yan and Kang, 2003; Yan et al., 2007; Wolde et al., 2018). Ideal environments tend to easily discriminate genotype performance providing a basis for selecting superior genotypes (Wolde et al., 2018). According to Yan and Kang (2003), the length of the vector is a measure of the environment's ability to discriminate the hybrids. Based on this, MKRS was the most discriminating and representative environment, while KRC was the least (Figure 5.3). The MKRS environment exhibited higher ability to discriminate genotypes in terms of their genotypic main effect during evaluation. The ideal environment provides suitable temperatures, humidity, and total rainfall manifesting in better genotypic performance. The results agree with Tonk et al. (2011) who reported that ideal environments allow genotypes to fully express their genetic potential enabling the identification of the best performing genotypes. Highly significant environmental effects could be explained by different altitudes, moisture and other stress conditions (Löffler et al., 2005).

Other environments had more or less similar discriminating ability, excluding STAP which had the second longest vector (Figure 5.3). Evaluating genomic predicted single cross hybrids at any of the optimal sites like MKRS, MPRS, RARS, ART and LWRS can help minimize breeding and testing costs by pinpointing a few top-performing genotypes prior to conducting extensive multi-environment trials. These results agree with Wolde et al. (2018) and Mafouasson et al. (2018) who employed GGE bi-plot analysis to identify ideal environments for enhancing genotype performance and maximizing the use of limited resources for testing. KRC and STAP were the least ideal, providing little discriminating information about the genotypes among the testing locations. The results agree with Tonk et al. (2011) and Wolde et al. (2018) who reported less-discriminating test locations which conferred undesirable effects on maize genotype performance. Test locations that are consistently less-discriminatory provide negative effect values on genotype performance (Yan et al., 2007). The reduced discriminatory power of an environment may be caused by abiotic and biotic factors, which can prevent genotypes from reaching their full genetic potential making identification of superior genotypes difficult (Yan and Tinker, 2006).

Conclusions

The study found the importance of combining ability in maize breeding, highlighting the significant general and specific combining ability effects of newly selected inbred lines on grain yield and component traits. The study identified superior inbred lines (CTL03, G17NL544, G16NL721, and GS-PL07) and high-performing hybrids (15AG163 x G16NL679, G15NL304 x G17NL642, and 15AG162 x G16NL679) based on their combining ability for grain yield. Furthermore, the study demonstrated the impact of genotype, environment, and genotype-by-environment interaction on grain yield, recommending the use of the AMMI model for efficient breeding. The clustering of test locations into two mega-environments and the identification of stable, high-yielding hybrids (G15NL304 x G17NL544 and 15AG162 x G17NL544) provide valuable insights for targeted cultivation in Zimbabwe and Zambia and similar agroecologies.

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General Overview and Implications of Study Findings

Introduction

Maize (*Zea mays* L., 2n = 2x = 20) is a vital crop globally, valued for food, feed, bioethanol production, and industrial raw materials. In Africa, maize is a staple food crop for millions of households. The productivity of maize under smallholder production conditions in Africa remains low (< 2 t/ha), compared to the global mean yield of 6 t/ha. Various challenges, including abiotic and biotic stresses, socio-economic constraints, and policy issues, contribute to low productivity. Seed Co Ltd is developing hybrid maize breeding using complementary and contrasting inbred lines to address the main productivity challenges. The new lines and experimental hybrids should undergo phenotyping and genotyping using high-resolution SNP markers for selection. Using phenotypic and genomic selection, maize breeders accelerate inbred line development, incorporating desired traits and creating high-performing single crosses and three-way hybrids. The new hybrids would be rigorously tested for yield gains and stability across multiple locations, ultimately leading to the release and commercialization of high-yielding varieties to boost maize productivity and economic returns. In light of this background, this chapter summarises the research objectives, major findings, and implications of the study, and recommendations for future research. The specific objectives of the study were:

(i) to assess a maize germplasm panel's genetic diversity and population structure comprising 182 founder lines and 866 derived inbred lines using Single Nucleotide Polymorphism markers to identify genetically unique lines for hybrid breeding.

(ii) to conduct genome-wide prediction of yield and component traits using qualitative and quantitative phenotypic traits and SNP markers based on the additive-dominant genomic best linear unbiased predictions model to compute genomic estimated breeding values and genomic estimated genetic values to guide inbred line development and hybrid breeding.

(iii) to assess the gains in yield and yield components among single cross maize hybrids selected through genomic prediction across representative locations to guide breeding and production and

(iv) to determine the combining ability effects of newly selected inbred lines and quantify the magnitude of heterosis and genotype by environmental interaction (GEI) effects of single cross hybrids to select and recommend contrasting elite lines and experimental hybrids.

Genetic diversity and population structure of tropical maize inbred lines using SNP markers.

A maize germplasm panel of 182 founder lines and 866 derived inbred lines was characterized for genetic diversity and population structure analyses using SNP markers to identify genetically unique lines for hybrid breeding through beneficial allelic combinations. Genotyping was performed using the Affymetrix platform for 182 founder lines (with 1201 SNP markers) and the Midseq platform for the 866 derived lines (1484 markers). The major findings of this study were:

- Moderate genetic variation was detected with genetic distances ranging from 0.004 to 0.44 (mean: 0.25) for the founder lines and 0.004 to 0.34 (mean: 0.13) for the derived lines.
- Heterozygosity values ranged from 0.00 to 0.24 for both lines.
- High polymorphism information content (PIC) was detected in 82% of SNP markers for founder lines and 84% for derived lines, indicating high genetic diversity.
- Analysis of molecular variance revealed significant genetic differences ($P \leq 0.001$) among and within populations; most detected variations at 97% and 88.38% were attributed to within populations in founder and derived lines, respectively.
- Population structure analysis identified three distinct subpopulations among founder lines and two among derived lines.
- Cluster analysis supported the population structure and delineated genetic relationships among tested populations.
- Genetically distant founder and derived inbred lines were selected based on pairwise comparison: G15NL337 and G15NL312 (Cluster 1), 15ARG152 and RGS-PL44 (Cluster 2), RGS-PL44 and 15ARG149 (Cluster 2) and RGS-PL33 and RGS-PL44 (Cluster 2).
- The selected lines are genetically distinct and recommended for marker-assisted hybrid maize breeding to exploit the frequency of beneficial alleles.

Genome-wide prediction of yield and component traits in maize using qualitative and quantitative phenotypic traits and genomic best linear unbiased prediction model.

1,102 genetically diverse inbred lines from two heterotic groups (N3 and SC) were genotyped using high-density SNP markers. 1102 lines and 4 testers were crossed using a line-by-tester design to generate 2830 single cross hybrids (SCHs). Phenotypic data were collected based on field trials using the following SCHs: 684 evaluated at five locations in 2018/19, 760 at four locations (2019/20), 646 at four locations (2020/21), and 740 at four locations (2021/22) summer seasons in Zimbabwe. The field trials were laid out in a 6 x 7 alpha lattice design with two replications at each site. The core findings of this study were:

- 20 high-performing and contrasting inbred lines with the highest genomic estimated breeding values (GEBVs) and genomic estimated genetic values (GEGVs), each from the two heterotic groups, were identified for genetic advancement, combining ability tests and commercial hybrid development.
- 20 high-performing candidate SCHs with high GEGVs were identified for three-way hybrid development, variety registration and commercialization.

Response of single cross maize hybrids selected through genomic predictions for yield and yield Components

Eleven elite inbred lines from two heterotic groups (N3 and SC) with the highest GEGVs for yield and associated traits were selected using genomic selection (GS) through the genomic best linear unbiased prediction (GBLUP) model. Six N3 lines used as female parents and five SC lines used as male parents were crossed using a factorial mating design to generate 30 SCHs. The 30 SCHs and six commercial single cross check hybrids used as comparative controls were evaluated in seven locations, four in Zimbabwe and three in Zambia, in the 2022/2023 season. The 36 hybrids were field evaluated using a 6 x 6 alpha lattice design with two replications at each location. The major findings of the study were:

- Significant genetic variation ($P<0.05$) was observed among the hybrids for the assessed 11 quantitive traits.
- Experimental hybrids CTL03 x G16NL721, 15AG162 x G16NL679, and G15NL304 x G17NL544 displayed the highest grain yield (GY) in Zimbabwe.
- Hybrids 15AG162 x G17NL544, CTL03 x G17NL544, GS-PL07 x G17NL544, and CTL03 x G16N721 recorded the highest GY in Zambia.
- Three top-performing SCHs (CTL03 x G16NL721, CTL03 x G17NL544, and GS-PL07 x G17NL544) displayed high and stable yields across all tested locations.

- Significant genetic gains were recorded for various agronomic traits. Gains for anthesis date (AD), silking date (SD), anthesis-silking interval (ASI), plant height (PH), ear height (EH), total lodging (TL), and moisture content (MOI), ranged from -27.82 to -0.18%.
- Positive genetic gains (0.05 to 8.88%) were recorded for the number of plants per 6m^2 (NP), number of ears harvested per 6m^2 (EHP), and number of ears per plant (EPP).
- Yield gains were significant, with a 13.09% increase over the mean of checks, 10.83% over the population mean, and 1.47% over the mean of the best check variety.
- Moderate to high broad-sense heritability (50-94%) and genetic advance as a percentage of the mean (GAM) (10-51.34%) was recorded for most traits.
- Positive and significant correlations ($P<0.05$) were found between GY and PH, EH, NP, EHP, EPP, and MOI.
- Negative and significant correlations were observed between GY and ASI ($r = -0.32$) and GY and TL ($r = -0.36$).
- Integrated phenotypic and genomic selection enhances genetic gains and may reduce the breeding cycles and costs of hybrid breeding in maize.

Combining Ability and Genotype by Environment Interaction Analyses among Single-Cross Maize Hybrids Developed Through Genomic Prediction.

Eleven elite inbred lines (6 female parents selected from N3 and 5 male parents from SC group) were crossed using a factorial mating design and 30 SCHs were developed. The lines had the highest GEGVs for yield and component traits and were selected using GS through the GBLUP model. The 30 SCHs and six commercial check hybrids used as comparative controls were evaluated under field conditions at seven locations, four in Zimbabwe and three in Zambia in the 2022/2023 summer season. The trials were laid out using a 6 x 6 alpha lattice design with two replications at each location. Data were recorded on yield, and yield components and general combining ability (GCA) and specific combining ability SCA) effects were computed. The main findings of the study were:

- Significant GCA effects for GY were recorded for lines CTL03, G17NL544, G16NL721, and GS-PL07.
- Significant SCA effects for GY were computed for crosses 15AG163 x G16NL679, G15NL304 x G17NL642, and 15AG162 x G16NL679.

- The additive main effects and multiplicative interaction (AMMI) model accounted for 38.95% of the total variation in grain yield due to genotype (G), 50.58% due to environment (E), and 7.24% due to genotype x environment interaction (GEI).
- The test locations were clustered into two mega environments consisting of Rattray Arnold Research Station (RARS), Agricultural Research Trust (ART), Mpongwe Research Station (MPRS) and Lusaka West Research Station (LWRS) (Environment 1) and Mkushi Research Station (MKRS), Stapleford Research Centre (STAP) and Kadoma Research Centre (KRC) (Environment 2).
- The genotype and genotype-by-environment interaction (GGE) biplot analysis identified hybrids G15NL304 x G17NL544 and 15AG162 x G17NL544 as high-yielding and stable, suitable for commercialization.
- The two mega-environments and the selected stable, high-yielding general and specific combiners are recommended for genotype evaluation and production in Zimbabwe and Zambia and similar agroecologies.

Implications of the findings in the practical breeding programs

- The study identified genetically unique inbred lines through genotyping with SNP markers. The distinct heterotic groups in the founder and derived lines will be used to select diverse parental lines for hybrid breeding.
- Significant genetic differences were found in the test populations (97% and 88.38% within the founder and derived lines, respectively) which can serve as a valuable resource for trait discovery. The results will enable the identification of unique traits within the highly differentiated populations to improve existing cultivars or create novel varieties.
- The study identified high-performing inbred lines with higher GEBVs and GEGVs from the N3 and SC heterotic groups. These are recommended for further crosses to maximize genetic gains in grain yield and yield-related traits.
- The study found significant genetic variation amongst the newly developed hybrids. This variation can be harnessed to create new hybrids with desirable characteristics, such as early flowering, improved plant height, increased yield, and enhanced grain quality.

- The study recommends SCHs, e.g., CTL03 x G16NL721, CTL03 x G17NL544, and GS-PL07 x G17NL544 to develop three-way hybrids with broad adaptability or for direct release as commercial varieties.
- The positive correlations between GY and PH, EH, NP, EHP, EPP, and MOI is useful for direct or indirect selection.
- The parental lines CTL03, G17NL544, G16NL721, and GS-PL07 were identified as good combiners for grain yield for future breeding.
- The study recommends hybrids with significant SCA effects, such as 15AG163 x G16NL679, G15NL304 x G17NL642, and 15AG162 x G16NL679, as prime candidates for commercialization and three-way hybrid breeding.