GENETIC ANALYSIS OF MAIZE GENOTYPES UNDER DROUGHT, LOW SOIL NITROGEN AND COMBINED STRESS, AND HERBICIDE REACTION OF SELECTED INBRED LINES

BY

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DECLARATION

I certify that this study represents my own original work, with due acknowledgement given to other researchers' work where cited. No portion of this study has been presented elsewhere for any degree or academic recognition.

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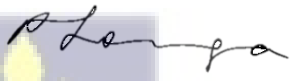

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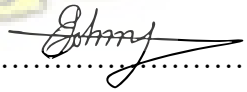

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ABSTRACT

The maize breeding programme at the West Africa Centre for Crop Improvement (WACCI) leverages genetic diversity from collected and developed parental lines to develop hybrids that would contribute to improved maize yields in Ghana. However, the performance of newly developed maize inbred lines across varying environments remains inadequately understood as there is limited information. Evaluating genetic diversity and its relationship to plant performance under varying environments is a crucial step for the identification of elite inbred lines with broad adaptability and resilience as well as the development of resilient hybrids for commercialization using these identified inbred lines. The objectives of this research included the following: i) assess the genetic diversity among selected white and yellow kernel maize inbred lines using field phenotyping and ISSR markers, ii) evaluate the response of maize inbred lines to nicosulfuron-based herbicide for potential selection of herbicide-tolerant inbred lines, iii) determine the gene action conditioning tolerance to drought, low soil N, combined drought and low soil N in maize inbred lines, and iv) assess the performance and stability of the hybrids under drought, low soil N, combined stresses, and optimum environments. One hundred maize inbred lines were evaluated under two optimal conditions - one with manual weed control and the other with chemical control using a nicosulfuron-based herbicide – to identify herbicide-tolerant lines. Based on *per se* performance, fifty inbred lines were subsequently selected and evaluated under drought conditions to identify drought-tolerant lines. The selected fifty lines were assayed using thirty inter-simple sequence repeats (ISSR) with only ten showing amplified bands. C316-7, DHL 258 POP 2, and TZIL 25 (60E) 0.4 EMS were consistently identified as superior inbreds across environments, showcasing their resilience and potential for use in breeding programmes with the objective of stress tolerance and yield stability. Inbred lines such as C316-7, DHL 258 POP 2, and DHL 99

POP 2 exhibited herbicide tolerance while DHL 101 POP 5, EXP 124 and TZIL 25 (35G) 0.4 EMS showed superior performance with high Yield Stability Index (YSI) and low Stress Susceptibility Index (SSI), confirming their potential as sources of drought tolerance. Molecular characterization using ISSR markers revealed high polymorphism among the 50 inbred lines, with ISSR-06 recording the highest Polymorphic Information Content (PIC = 0.78), revealing substantial genetic diversity and clutering of inbred lines into four distinct clusters. Twenty-four maize inbred lines were selected and used to generate ninety-six single cross hybrids using the North Carolina Design II (NCD II). The hybrid trials were evaluated at two locations, Legon and Kwadaso, under low soil nitrogen (N), induced drought, combined drought and low soil nitrogen and optimal environments in 2023 and 2024. There was preponderance of additive gene action (GCA) for most traits across environments indicating that recurrent and pedigree selection would be effective for yield improvement and stress tolerance. However, notable non-additive gene action for yield components such as number of ears per plant and ear aspect, highlight the importance of heterosis breeding in exploiting specific parental combinations. Hybrids such as LMI 102 X LMI 140, HP020-4/1(A)/1/1 X DHL 46 POP 1, 1368 X DHL 186 POP 2 and TZIL 25 (40C) 0.4 EMS X TZIL 25 (60E) 0.4 EMS were the superior hybrids in different stress environments, while DHL 99 POP 2 x 9006 and DHL 99 POP 2 x EXP 124 demonstrated broad adaptability across multiple environments. GGE biplot analysis identified three mega-environments and revealed that LMI 102 X LMI 140 was the highest-yielding across all the environments but with moderate stability, whereas DHL 99 POP 2 x 9006 and 1368-150GY(119)S x DHL 184 POP 2 were the most stable, making them ideal for wide adaptation. Superior hybrids identified should be further tested in multiple environments. These can be recommended for location- and stress-specific conditions.

DEDICATION

To God Almighty, my ever-present help, guide and source of strength.

To my wonderful children, Eyram and Mawusi, for being my greatest source of motivation, inspiration and joy.

To my parents, whose love, sacrifices and unwavering belief in me have formed the foundation of my journey paving the way for my achievements.

And to my siblings, for their constant support and encouragement.

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To my family-my incredible parents, supportive siblings and beloved children-you have been my anchor and greatest source of motivation. Your unwavering love, support, encouragement and sacrifices have made all the difference.

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LIST OF ABBREVIATIONS

BLUP	Best linear unbiased prediction
CIMMYT	International Maize and Wheat Improvement Centre
DAS	Days after spraying
DASS	Days after second spraying
DHL	Double haploid line
GCA	General Combining Ability
GEI	Genotype x Environment interaction
HGCAMT	Heterotic grouping based on the GCA of multiple traits
IITA	International Institute of Tropical Agriculture
ISSR	Inter simple sequence repeat
MAS	Marker-assisted selection
NCD II	North Carolina Design II
QTL	Quantitative trait loci
SCA	Specific Combining Ability
SSA	sub-Saharan Africa
SSI	Stress susceptibility index
UPGMA	Unweighted pair group method with arithmetic average
WACCI	West Africa Centre for Crop Improvement
YSI	Yield susceptibility index

CHAPTER ONE

1.0 INTRODUCTION

Maize (*Zea mays*) is the most important staple food crop produced and consumed by majority of the populace in sub-Saharan Africa (SSA), sustaining livelihoods as well as playing a crucial role in the economy. This adaptable crop is cultivated under diverse climatic and agro-ecological conditions contributing significantly to food and nutrition security (van Zonneveld *et al.*, 2023) in SSA. Maize provides a reliable source of calories, carbohydrates, and essential nutrients, serving as a mainstay in the diets of millions of people in the sub-region (Mdoda *et al.*, 2021; Mthembu *et al.*, 2022). In Ghana, maize is the principal staple crop, predominantly cultivated by smallholder farmers and rural communities mostly under rain-fed conditions. The crop thrives across most of the ecological zones of Ghana, including the Northern Savannah, which receives the lowest amount of rainfall in both distribution and quantity (Mustapha *et al.*, 2020; Yagle *et al.*, 2024) and ranks first in terms of area cultivated exceeding one million hectares, representing approximately 50-60% of the country's total cereal production (Obour *et al.*, 2022). As a staple crop, maize provides economic opportunities and strengthens cultural ties in many communities (Noxolo Nesamvuni *et al.*, 2022; Ouédraogo *et al.*, 2023; Raphela & Pillay, 2021). Maize production is a major source of food for most Ghanaians and very important for ensuring Ghana's household food security. The crop is also an integral part of poultry and livestock feeds and a substitute for the brewing industry.

Despite its significant economic benefits and adaptability across most ecological zones in Ghana, the country remains one of the lowest producers of maize on the continent with South Africa leading production at over 16.8 million tons, followed by Nigeria, Ethiopia and Egypt (FAOSTAT, 2023). In Ghana, maize yields average 1.7 metric ton/ha (Asante *et al.*, 2020) to 1.99 metric ton/ha

(Danquah *et al.*, 2020) well below the potential yield of 6 metric ton/ha achievable with improved varieties under optimal conditions (Danquah *et al.*, 2020). This wide yield gap is attributed to the combined effects of multiple stresses that occur concurrently on farmer fields, thereby limiting maize productivity. Increasing temperatures, extended dry spells, among a host of other biotic and abiotic stresses, such as low soil fertility, the fall army worm and the parasitic weed, *Striga hermonthica,* severely constrain productivity in SSA, (Dojamo *et al.*, 2022; Gichile, 2022; Njeru *et al.*, 2023) including Ghana.

Among the many environmental conditions that affect maize production, recurrent drought, which is because of inadequate rainfall and poor distribution, is one of the major environmental stresses that affects maize production leading to lower yields being recorded on most farmers' fields. With the onset of global climate change, the risk of frequent drought is expected to intensify, especially in rain-fed agricultural systems (Christian *et al.*, 2023; Cook *et al.*, 2020; Khadka *et al.*, 2024). Kasei *et al.* (2010) emphasized that while extreme climatic events such as floods occur sporadically, drought frequency is on the rise. Issahaku *et al.* (2016) reported seasonal and decadal variations in rainfall in the Upper East Region, indicating a potential reduction in average rainfall between 2.8% and 10.9% by 2050. This is corroborated by Osman (2023) who noted the increasing frequency of drought with about 98% of farming households reporting climate hazards such as drought, erratic rainfalls, and extreme temperatures, which threaten food security and livelihoods. Since the introduction of the *'Planting for Food and Jobs (PFJ)'*, in 2017, maize yields in Ghana have steadily increased to above 2 tonnes/ha recording a highest in 2022 at 2.6 tonnes/ha (FAOSTAT, 2023). This increase is attributed to the introduction of subsidized prices of hybrid seed as well as agro-inputs such as fertilizer through the PFJ initiative. However, by 2023, maize yields had plummeted to 0.9 tonnes/ha, significantly lower than the global average of 5.9 t/ha

(FAOSTAT, 2023). The decline in 2023 was primarily due to long drought spells experienced during the major rainy season that affected many crop-growing regions across the country, highlighting the increasing impact of climatic conditions.

Drought and heat stress are the most limiting environmental stresses on maize productivity resulting in estimated yield losses of 15-20% annually (Meseka *et al.*, 2018; Hussain *et al.*, 2019; Yousaf *et al.*, 2022; Yawale *et al.*, 2023). Drought affects maize growth and development at multiple stages, but its occurrence during the reproductive stage is the most destructive (AbdElgawad *et al.*, 2020; Liu *et al.*, 2021). It can hinder seedling establishment, restrict vegetative and root growth, reduce photosynthetic activity, prolong, anthesis-silking interval, and disrupt pollination and grain formation (Daryanto *et al.*, 2016; Ji *et al.*, 2017; Zhang *et al.*, 2018; Waititu *et al.*, 2021). Drought occurrence two weeks before and during the anthesis-silking stage reduces seed set and kernel size, often resulting in yield losses of 20-50% or complete crop failures in severe cases (Alioum *et al.*, 2020; Maheswari *et al.*, 2021; Iseghohi *et al.*, 2022). These losses are largely attributed to delayed silking relative to anthesis, which causes poor synchronization with pollen shed and leads to poor fertilization and barren cobs. Post-Disaster Needs Assessment (PDNAs) conducted between 2007 and 2022 indicate that agricultural losses accounted for an average of 23% of the total disaster impact across all sectors, with more than 65% of drought-induced losses borne by agriculture. Cereal crops were particularly vulnerable, with global losses averaging 69 million tonnes annually over the past three decades (FAO, 2023). Between 2006 and 2018, drought was identified as the single greatest driver of production loss in Africa, accounting for over 80% of total agricultural losses. Cereals, particularly maize, were the most affected commodity (FAO, 2021). These findings underscore the extreme vulnerability of African agriculture, particularly, maize to drought.

Most soils in sub-Saharan Africa are tropical and inherently low in fertility, particularly in nitrogen and organic matter. This nutrient depletion is further aggravated by continuous cultivation, low fertilizer application rates, and poor soil management practices. Among the essential nutrients, nitrogen, a major requirement for increased levels in maize yield, is the most limiting factor for maize production, constraining yield potential across the region. Nitrogen plays a key role in determining the grain yield through the role it plays in photosynthesis and other biological processes such as mineral and water absorption, vacuole storage, and transport in the xylem cells (Guo *et al.*, 2021; Sun *et al.*, 2022 Gao *et al.*, 2023; Kafle *et al.*, 2023). Meng *et al.* (2022) found that the application of nitrogen fertilizer can increase maize yield by 184.9% to 193.7% when applied at optimal rates, highlighting the critical role of nitrogen in maize production. Similarly, Wang *et al.* (2023) reported that appropriate fertilizer application, particularly nitrogen and phosphorous, enhances dry matter accumulation and grain yield, indicating that higher fertilizer rates correlate with improved maize productivity. However, due to unavailability and high prices of inorganic fertilizer for most resource-poor farmers, rates of fertilizer application are still lower than the recommended dosage. Hickman *et al.* (2015) noted that most recommended fertilization rates for maize in smallholder agriculture are below the 100 kg/ha threshold, which is considered ideal for achieving substantial yield increases. In Ghana, nitrogen use per area of cropland reported by FAOSTAT (2023) was 10.48 kg/ha, 8.13 kg/ha for phosphate and 4.92 kg/ha for potash. The estimated annual loss of maize yield in the sub-region due to low soil nitrogen varies from 10-50% (Badu-Apraku *et al.*, 2016; Ajala *et al.*, 2018; Aboderin *et al.*, 2023).

Due to the rapid and dynamic global environmental changes, plants grown under the field conditions mostly face a combination of various abiotic stresses, such as drought and suboptimal or low soil fertility. On most farmer fields, several abiotic stresses such as recurrent drought, and

low soil fertility occur simultaneously or at different stages of crop growth, and the combined effect is devastating on cereal crops especially maize. Research studies have reported these stresses as important stress factors that inhibit maize productivity and affect the livelihoods of farmers (Cairns *et al.*, 2013; Cohen *et al.*, 2021;Rafique, 2021). Several studies in crops involving abiotic stresses have traditionally addressed individual stresses in isolation (Singh *et al.*, 2017; Obeng-Bio, 2018; Abu *et al.*, 2021; Karande *et al.*, 2022; Malik *et al.*, 2020; Martey *et al.*, 2023), while in recent times there has been increased focus on understanding crop adaptability in the presence of two or more stresses (Cairns *et al.*, 2013; Cohen *et al.*, 2021; Rafique, 2021). Studies conducted in various crops have revealed that plant responses to combined stresses are distinct and cannot be directly inferred from their responses to individual stresses applied separately (Rizhsky *et al.,* 2002, 2004; Vescio *et al.*, 2021). The co-occurrence of more than one stress, such as drought and low soil fertility, has been shown to exert synergistic negative effects, leading to severe declines in plant growth and survival (Mittler, 2006). This reality underscores the importance of breeding maize varieties that can tolerate the combination of abiotic stresses commonly experienced in farmers' fields, rather than single stresses in isolation.

To achieve this, it is essential to understand the genetic mechanisms that underline maize responses to multiple stresses. Due to the distinctive response of plants to combined stresses, effective breeding requires insights into the nature of gene action and the ability to identify parental lines and cross combinations that perform reliably under stress combinations. A genotype's performance is determined by its genetic composition and the environment in which it is cultivated, with trait expression influenced by additive, dominance, epistatic, and over-dominance effects (Abu *et al.*, 2021; Viana, 2022; Viana & Garcia, 2022; Kimutai *et al.*, 2023; Loro *et al.*, 2023; Bourandi *et al.*, 2024). The extent and direction of genetic components are assessed using various

parameters including combining ability, heritability and heterosis analyses. Understanding gene action and its associated trait expression is crucial for effective breeding and selection as it enables the breeder to determine which breeding strategy to apply for targeted quantitative traits (Sunday *et al.*, 2020; Bhadmus *et al.*, 2021; Nyombayire *et al.*, 2021;).

Combining ability studies play a crucial role in designing plant breeding programmes, enabling breeders to choose appropriate breeding methodologies for trait improvement (Han *et al.*, 2020; Bocianowski *et al.*, 2023; Karjagi *et al.*, 2023). These are extensively utilized in maize breeding programmes to assess general combining ability (GCA) and specific combining ability (SCA) effects of maize germplasm. GCA and SCA are the two concepts that have had important influence on inbred line evaluation and population development in crop breeding (Obeng-Bio *et al.*, 2020; Adu *et al.*, 2021; Amegbor *et al.*, 2022; de Faria *et al.*, 2022). These analyses help identify the nature of gene action governing the expression of quantitative traits, evaluate genetic diversity, and classify heterotic groups and patterns thereby guiding the development of superior hybrids (Noor, 2015; Elmyhun *et al.*, 2020; Zandalinas & Mittler, 2022). Studies have shown that individual performance of inbred lines is not always a reliable indicator of their superior combining ability. Certain plant cross combinations may yield superior hybrids due to favourable gene interactions, while others involving promising parents may result in progeny that are inferior. Thus, systemic evaluation of germplasm through combining ability analyses is essential for identifying both superior parental lines for breeding programmes and favourable hybrid combinations (Chen *et al.*, 2019; Han *et al.*, 2020b; Han *et al.*, 2020; Hemavathy *et al.*, 2023; Hilli *et al.*, 2020; Liu *et al.*, 2021).

Amiruzzaman *et al.* (2013) conducted a diallel analysis among elite maize inbred lines, highlighting how GCA and SCA can be utilized to assess the nature of gene action and to identify

superior combiners that can be hybridized to exploit heterosis. Similarly, Elmyhun *et al.* (2020), reported significant variability in GCA and SCA among maize inbred lines, indicating the presence of genetic variability that can be harnessed for breeding purposes. Hence, evaluation of inbred lines for combining ability and heterosis is an important component of maize hybrid breeding program.

In parallel, it is also imperative that the genetic diversity present among inbred or parental lines be determined to ensure that the selection of parental lines for hybrid development targets lines with high genetic dissimilarity for increased heterosis (Govindaraj *et al.*, 2015; Nyomabayire *et al.*, 2016). With the use of molecular markers, polymerase chain reaction (PCR)-based approaches are commonly used to identify and assess genetic diversity in plant germplasm. Inter simple sequence repeat (ISSR) markers are highly polymorphic markers that are regarded as reproducible and specific tools for studies on genetic diversity, phylogeny, genetic coding, genomic mapping and evolutionary biology (Ahmed *et al.*, 2022; Aji *et al.*, 2022; Rini *et al.*, 2023). Yani *et al.* (2022) highlighted significant genetic variability in eight maize cultivars from East Nusa Tengrara, Indonesia, showcasing the ability of ISSR markers to reveal genetic relationships among different maize genotypes. The high polymorphism rates associated with ISSR markers exceeding 80% in many studies, indicates their effectiveness in distinguishing between closely related genotypes (Wahyudi *et al.*, 2020; Yani *et al.*, 2022).

In the ever-changing climate and environment, plants have adapted to survive under the myriads of stress factors that occur simultaneously. Due to the immobile nature of plants, specific mechanisms have been developed by plants to enable them to detect environmental changes and adapt to varying stress conditions, thereby minimizing damage while preserving essential resources for growth and reproduction. In the occurrence of a combination of multiple stresses,

plants exhibit distinct physiological and molecular adaptations that differ from those triggered by individual stressors (Anwar *et al.*, 2021; Zandalinas & Mittler, 2022). Hence techniques for developing and testing stress-tolerant varieties by imposing each stress individually may be insufficient and inefficient.

The specific objectives of this research include the following:

i) characterize the performance and genetic diversity of selected WACCI white and yellow kernel maize inbred lines across contrasting environments using morphological traits and ISSR markers,

ii) evaluate the performance and stability of the hybrids under drought, low soil N, combined stresses, and optimum environments, and

iii) determine the gene action governing tolerance to drought, low soil nitrogen and combined drought and low soil nitrogen in maize inbred lines.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Maize production

Maize (*Zea mays* L.), also referred to as corn, is a cross-pollinated crop in the Poaceae (Gramineae) family. It is a monoecious plant with imperfect flowers, that is, separate male and female flowers on the same plant. Maize originated from Central America and Mexico and was domesticated from the teosinte *Z. mays* subsp. *parviglumis*. It has several types including flint, dent, flour, popcorn and sweet corn. Globally, maize is one of the most significant cereal crops, serving as a staple food and a critical component of food security, particularly in sub-Saharan Africa (SSA). This crop is not only utilized for human consumption but plays a vital role in livestock feed and industrial applications.

Over the past few decades, maize has experienced substantial growth across the world. In 2021, the total production reached approximately 1.2 billion metric tons, with the United States, China and Brazil being the leading producers (Matimolane *et al.*, 2022). The United States accounted for about 30% of global maize production, attributed to the utilization of advanced agricultural technologies and extensive land dedicated to maize production. China, follows closely, with significant production driven by domestic demand for both human consumption and animal feed (Chemura *et al.*, 2022). In Ghana, maize production has been reported to exceed 3 million metric tons in recent years, reflecting a rising trend since 2016 (Gyamerah *et al.*, 2024). However, projections indicate that maize yields could decline by approximately 20% by 2050 due to climate change impacts (Kyei-Mensah *et al.*, 2019).

2.2 Relationship between drought and low soil nitrogen and its effect on maize productivity

Agricultural drought stress occurs when plants experience a water deficit, resulting in reduced water uptake and increased water loss through transpiration, thereby leading to crop failures severely affecting food security across the globe. The impact of drought stress depends on the interaction of plant, environmental, and management factors: the crop developmental stage, rate of water deficit, peak intensity of the deficit, and planting density (McMillen *et al.*, 2022). Drought occurrence during the reproductive/flowering stage can be very detrimental to the crop as it causes a delay in silk emergence thereby leading to an increase in the anthesis-silking interval, which is an important cause of yield reduction in the crop. Drought disrupts plant growth, nutrient and water balance, photosynthesis, and assimilate partitioning, ultimately leading to significant decline in crop yields (Du *et al.*, 2020; Tiwari *et al.*, 2022; Kalra *et al.*, 2023;Fatima *et al.*, 2024).

Different molecular, biochemical, physiological, morphological and ecological traits and processes (Figure 2.1) of the plants are impaired under drought stress conditions (Ortiz *et al.*, 2015). Typically, visual symptoms of drought include change in leaf colour from green to pale green, the rolling of leaves and stunted growth with crop failure in the occurrence of severe and prolonged drought. These symptoms can be attributed to various morphological and physiological responses to the occurrence of drought. Drought impairs mitosis and cell elongation, leading to stunted plant growth (Kim *et al.*, 2020; Lephatsi *et al.*, 2022; G. Sun *et al.*, 2023). It also restricts cell growth due to the loss of turgor (Taiz and Zeiger, 2006). Limited leaf expansion under drought conditions is because of reduced turgor pressure and slow rate of photosynthesis (Coussement *et al.*, 2021; He *et al.*, 2023). Other physiological responses include stomatal closure, reduced transpiration, and increased leaf temperature.

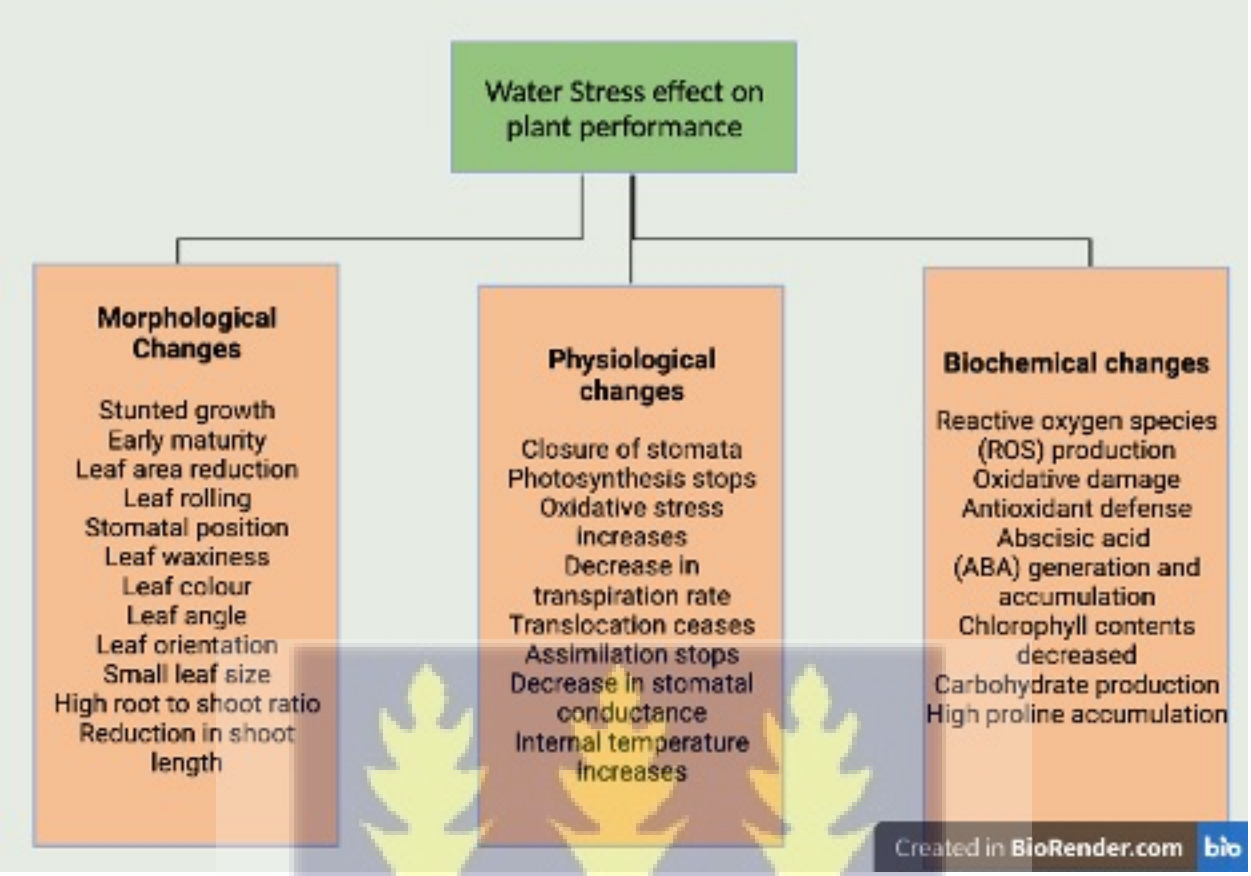

Figure 2. 1: Morphological, physiological, and biochemical dynamics of plants affected by water stress (Seleiman *et al.*, 2021).

Soil nutrient deficiency, with nitrogen being the most limiting nutrient, is a major production constraint in maize especially in smallholder farms in sub-Saharan Africa, including Ghana (Assefa & Mekonnen, 2019; Azumah *et al.*, 2022). Farmers in the sub-region apply less than 10 kg per hectare (ha) in their maize fields thus experiencing low grain yields regardless of the variety cultivated and the crop management techniques applied (Badu-Apraku *et al.*, 2023). Under low soil nitrogen (N) stress, maize varieties are known to perform poorly. This is attributed to the crop needs of nitrogen for its growth and productivity. An optimum supply of N fertilizer increases total biomass production and crop yield but at lower N fertilizer supply, plant dry matter accumulation

of reproductive parts decreases, resulting in lower grain yield (Nehe *et al.*, 2018; Hammad *et al.*, 2022; Zhang *et al.*, 2022). Low soil nitrogen reduces the photosynthetic capacity of the plant due to impaired leaf development resulting in reduced leaf area and subsequent leaf senescence. Cechin *et al.* (2022) demonstrated that plants grown under low nitrogen conditions exhibit a significant decrease in stomatal conductance and photosynthesis, with total leaf nitrogen content significantly lower than in high-nitrogen conditions. Research by Ige *et al.* (2021) indicates that low nitrogen availability negatively impacts the growth parameters of maize, including leaf area, which is directly correlated with photosynthetic capacity. Limited levels of nitrogen in the soil for plant assimilation affects protein synthesis, enzymatic action and hormones required for various metabolic process in maize plants (Figure 2.2).

The combination of drought and nitrogen stress (CDNS) has a synergistic negative effect on maize productivity. Drought occurrence in fields reduces nutrient uptake by the roots because the low soil moisture results in a decreased rate of diffusion of nutrients from the soil matrix to the absorbing root surface (Bista *et al.*, 2018; Lotfabadi *et al.*, 2022; Afzal *et al.*, 2023; Ebrahimi, 2023). Research has demonstrated that the interactive effects of drought and low soil nitrogen result in reduced stomatal conductance and leaf area thereby reducing photosynthetic efficiency, decreased biomass accumulation (Ali and Golembek, 2016; Studer *et al.*, 2017), and ultimately, decreased grain yield. In assessing the interactive effects of nitrogen, phosphorous and potassium-nutrition and drought stress on maize seedling development, Studer *et al.* (2017), found that the application of nutrients under deficient conditions could increase the drought tolerance of plants by increasing plant biomass for early growth vigour and establishment.

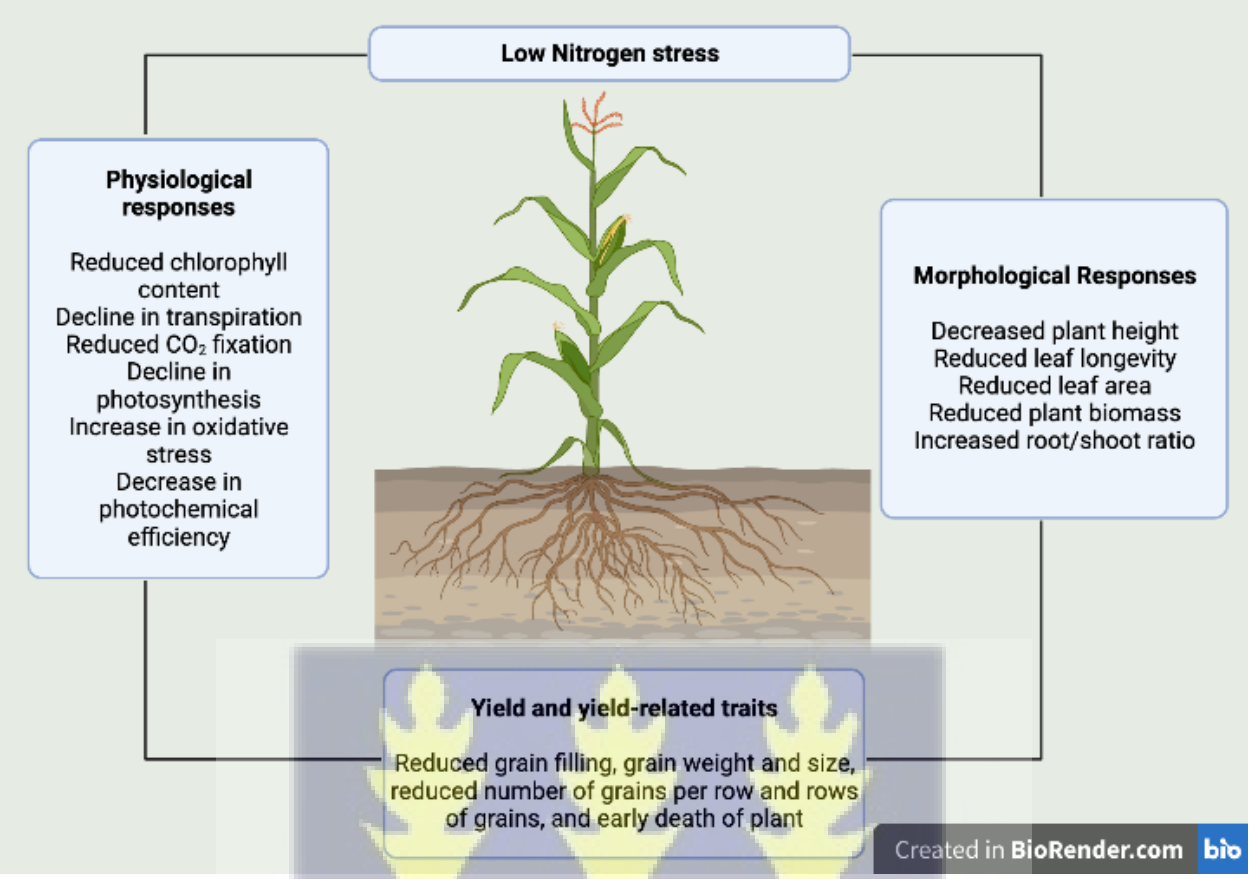

Figure 2. 2: Morphological and physiological dynamics of plants affected by low soil nitrogen stress (Santos *et al.*, 2023).

Similarly, Ao *et al.* (2020) reported that drought stress negatively affected the photosynthetic rate and leaf area in maize, especially when combined with low nitrogen levels. The evidence from these research findings, (Shi *et al.*, 2018; Wu *et al.*, 2022 Sharma *et al.*, 2023; Wang *et al.*, 2023), elucidates the intricate relationship between drought-induced stress and the dearth of soil fertility, specifically the insufficient presence of essential nutrients such as nitrogen, in influencing the developmental capacity of maize crops with a subsequent effect on yield.

Most studies in the sub-region on maize stress resilience have focused on single stressors, such as drought, low N or heat, leaving a significant gap in understanding the impact of simultaneous

stresses on maize. Moreover, research on combined stress condition has primarily focused on drought and heat, with limited work addressing the combination of drought and low N. Investigating the combined effects of drought and low N offers the potential to identify resilient maize varieties under these conditions and will contribute to a deeper understanding of how these stresses influence plant growth and yield.

2.3 Combining ability studies in maize breeding for stress tolerance

2.3.1 Overview of combining ability studies and its significance in maize breeding programmes

With the ever-increasing global population and the need for sustainable food production, maize breeding programmes play a critical role in developing resilient and high-yielding maize varieties. One of the key strategies utilized in maize breeding is combining ability analyses, which provides valuable insights into the genetic mechanisms governing the inheritance of traits and performance of parental lines and their progeny, assisting breeders to choose suitable parental lines for further crop improvement. Therefore, the understanding and utilization of combining ability among diverse maize germplasm continues to remain critical in the development and success of maize breeding programmes.

Combining ability, in the context of plant breeding, can be defined as the ability of parental lines of a crop to combine well with other crops of similar species during hybridization such that favourable genes or traits can be transmitted to their progenies. Sprague and Tatum (1942) introduced the concepts of general combining ability (GCA) and specific combining ability (SCA) which have played a pivotal role in the evaluation of inbred lines and the development of crop breeding populations (Griffing, 1956). GCA is defined as the average performance of a genotype in a series of hybrid combinations in parity to other inbred lines in similar hybrid combinations.

SCA is defined as the performance of a genotype in respect to certain hybrid combinations, that is, in the cases where hybrid combinations perform better or poorer than would be expected based on the average performance of the parental lines. GCA is largely due to additive gene actions while SCA is largely due to non-additive gene actions (dominance and epistasis). This information is valuable for breeders as it aids them to understand the underlying genetic mechanisms of trait expression and assists in determining effective breeding strategies while exploiting heterosis, also known as hybrid vigour.

The primary objective of combining ability studies is to predict the performance of hybrid combinations and identify superior parental lines with high combining ability for specific traits of interest. In maize, this has been extensively applied with several studies highlighting the importance of GCA and SCA in guiding hybrid development under both optimal and stress environments. Popović *et al.* (2025) demonstrated that maize landraces used in the study exhibited significant GCA effects for yield and grain nutritional quality attributes, providing opportunities for broadening the genetic base while improving nutritional value. Similarly, Matongera *et al.* (2023) highlighted the relevance of GCA in identifying zinc-donor lines that combine well with provitamin A, quality protein maize (QPM), and normal testers, thus accelerating progress in biofortification breeding without compromising agronomic performance. Amegbor *et al.* (2023) further identified inbred lines with favourable GCA effects across diverse environments as valuable resources for enhancing grain yield and quality, while emphasizing the differential contribution of additive versus non-additive effects to these traits. Complementing GCA, SCA estimates provide insights on specific cross combinations that exhibit superior performance for traits allowing for selection. Kamara *et al.* (2020) demonstrated the predictive value of SCA by evaluating twenty-eight (28) F_1 maize hybrids under varying plant densities. Yu *et al.* (2020)

identified hybrids from heterotic groups that contributed to highly significant positive SCA, potentially useful for commercial maize breeding. Their findings also showed that comparatively, there was a stronger correlation between heterosis and SCA than GCA, suggesting that SCA could serve as a reliable predictor of heterotic response in hybrid maize breeding. The identification of GCA of parental lines and SCA effects of crosses as well as understanding the genetic basis of specific traits of interest allows the breeder to make informed decisions in the selection of best-performing parental lines for hybrid development and the designing of breeding programmes to improve maize varieties.

2.3.2 Utilization of combining ability studies to develop stress-tolerant maize hybrids

Developing stress-tolerant maize hybrids is essential for ensuring stable crop production and food security. In the development of hybrids, two main steps are recognized: the development of inbred or parental lines and the selection of best parental lines for the exploitation of heterosis to develop elite hybrids. The *per se* performance of parental lines cannot be used to predict the yield performance of hybrids developed (Nyaga *et al.*, 2020; Guo *et al.*, 2024). On the contrary, it is largely controlled by the potential of the inbred or parental lines to combine well to create superior or elite hybrids (Qi *et al.*, 2013). Combining ability studies, which assess the genetic effects of parental lines and their crosses, provides valuable insights into the development of stress-tolerant maize hybrids.

GCA is a valuable tool for selecting parental lines based on performance of their progenies, usually the F_1 but it has also been applied in F_2 and later generations (Fasahat *et al.*, 2016). Inferences for selection of target traits can be made based on the value of the GCA or SCA. A low GCA value, positive or negative, implies that the mean of a parent used in a cross with another does not differ largely from the general mean of the crosses (Fashat *et al.*, 2016). However, a high and positive

GCA value shows the superiority of the parental mean to the general mean indicating a high possibility of gene flow from parental lines to progenies. It also indicates higher heritability and less gene-environment interactions. Inferences to be made from a negative or positive GCA value, whether low or high, is dependent on the trait of interest. For maize genotypes to be productive under stress environments, positive GCA effects for grain yield is essential. Choosing a parental line solely based on its SCA effect has limited usefulness in breeding programs (Fasahat *et al.*, 2016). Hence, SCA effect should be considered in combination with a high performing hybrid and should involve at least one parent with high GCA to maximize breeding efficiency (Dosho *et al.*, 2021; Yadesa *et al.*, 2021; Awata *et al.*, 2022; Karim *et al.*, 2023).

Understanding the proportion of additive and dominance variance components is crucial for developing an effective breeding strategy to improve a target trait (Ertiro *et al.*, 2017). Several studies in maize confirm that while grain yield often exhibits substantial dominance variance, reflecting the importance of heterosis in hybrid performance, additive variance provides the essential basis for genetic gain. Rogers *et al.* (2021a) reported that additive and dominance variance contributed equally to hybrid grain yield across diverse environments, whereas traits such as days to flowering were largely controlled by additive effects. Under drought and low nitrogen conditions, other studies have highlighted additive gene action as the main determinant of grain yield and other related traits (Barreto *et al.*, 2024; Sunday *et al.*, 2020b). In contrast, non-additive effects become more relevant under optimal conditions or for traits where heterosis can be maximized, as shown in the studies of yield and disease resistance (Ghazy *et al.*, 2024). With the introduction of genomic selection, models that incorporate both additive and dominance effects have improved the prediction of hybrid performance under stress (Barreto *et al.*, 2024). To optimize breeding efforts, it is recommended for breeding programmes to evaluate parents for

GCA and subsequently test resulting hybrids in target environments, considering that the genetic basis of GCA and SCA effects differs.

Despite the varying results in the understanding of the inheritance for certain traits under multiple stress environments, with some showing the preponderance of additive variance over non-additive variance and vice versa, combining ability studies have proven to be valuable in the development of stress-tolerant maize hybrids.

2.4 Breeding maize for combined abiotic stress tolerance: Challenges and Prospects

In the tropics of sub-Saharan Africa (SSA), maize is predominantly cultivated under rainfed conditions by resource-constrained smallholder farmers, often threatened by multiple biotic and abiotic constraints. These challenges are further exacerbated by climate change undermining the progress being made to improve the food and nutrition security as well as the livelihoods of millions of maize dependent smallholder farmers (Prasanna *et al.*, 2021). Improving crop productivity and livelihoods of smallholder farmers in the face of increasing climate variability, amidst low soil fertility, requires a multi-disciplinary approach to crop genetic improvement, with drought and low soil nitrogen stress identified as the major constraints to maize productivity (Hansen *et al.*, 2019).

Several studies have shown that drought not only affects maize yield directly but also interacts with other abiotic stresses, such as low nitrogen availability and heat, to further diminish crop productivity (Makumbi *et al.*, 2011; Meseka *et al.*, 2018; Annor *et al.*, 2019; Ribeiro *et al.*, 2020; Tufail *et al.*, 2021). This indicates a critical gap in breeding programmes, which often address single stress factors in isolation without considering the complex nature of multiple, co-occurring stress environments. Moreover, the genetic basis for combined stress tolerance remains

underexplored, while the physiological responses of genotypes to combined stresses are complex and not fully understood. Stutts *et al.* (2016) observed that the interaction between drought and low soil nitrogen does not produce additive effects; rather the response pathways are selectively activated. This complexity requires detailed research to understand the physiological mechanisms which would need comprehensive physiological assessments during trials to identify genotypes that can tolerate multiple stresses effectively. Also, Malenica *et al.* (2021) emphasized that research on simultaneous stress factors, such as Striga, low soil nitrogen, and drought is scarce. Findings from their study suggest that identifying tolerant genotypes to combined stresses is vital for improving tropical maize germplasm.

Over the past few decades, significant genetic gains have been achieved through conventional breeding methods that harness natural genetic variation within plant populations. These methods such as hybridization, selection, polyploidy, and introgression through backcrossing, have been central to developing stress-tolerant genotypes and ensuring yield stability across environments and locations (Afzal *et al.*, 2023). Collaborative efforts by the International Maize and Wheat Improvement Centre (CIMMYT) and the International Institute of Tropical Agriculture (IITA), in partnership with national programmes and the private sector, have further advanced this progress by using such conventional breeding approaches to develop climate-resilient maize varieties adapted to tropical environments in SSA. In 1997, the African Maize Stress (AMS) project, a CIMMYT-IITA partnership, established screening facilities that enabled the selection of drought-tolerant open-pollinated varieties (OPVs) and inbred lines for breeding in West and Central Africa. (Cairns *et al.*, 2013; Adebayo *et al.*, 2014). Acknowledging the complex genetic nature of drought tolerance, other initiatives such as the Drought-Tolerant Maize for Africa (DTMA), Stress-Tolerant Maize for Africa (STMA), and the Accelerated Genetic Gain projects, expanded breeding

efforts using conventional and molecular tools (Menkir *et al.*, 2024). In Ghana, these initiatives have strengthened maize breeding pipelines in institutions such as the Council for Scientific and Industrial Research-Crops Research Institute and Savanna Agricultural Research Institute, and the West Africa Centre for Crop Improvement (WACCI) and facilitated the release and commercialization of high-yielding, stress-resilient maize varieties through private seed sector partnerships.

Despite the progress made, conventional breeding approaches remain limited in their ability to adequately understand and address the complex nature of stress interactions. Also, the lack of comprehensive phenotyping and genotyping approaches that consider combined stress factors presents a significant barrier to progress. Hence the need to combine conventional breeding with modern techniques, which has proved to be a more valuable approach in the development of stress-resilient crops. The application of modern breeding techniques has contributed significantly to understanding and enhancing abiotic stress tolerance in maize. These techniques such as marker-assisted selection (MAS) and genomic selection have been used by breeders to accelerate the breeding process and improve the efficiency of selecting for abiotic stress tolerance. Conversely, modern breeding techniques, specifically MAS, offer a significant potential to accelerate the identification of plant populations exhibiting traits related to abiotic stress tolerance. Marker-assisted selection technique has been employed for the screening of germplasms, fingerprinting, QTL mapping and tagging of genes bestowing tolerance to abiotic stresses (Gantait *et al.*, 2019).

In maize, molecular markers have played a pivotal role in assessing genetic diversity and the development of superior hybrids. This is achieved by detecting and exploiting DNA markers associated with desirable traits, which are distributed across the genome. An additional advantage is that these markers can be readily detected at various stages of plant growth, independent of

environmental conditions. Hence, stress-resistant plant populations can be efficiently screened, even during the seedling stage, thereby saving time, space, and resources. Adeyemo & Omidiji (2019) analyzed the genetic diversity among farmers' maize varieties in Nigeria using simple sequence repeat (SSR) markers, demonstrating the relationship between local cultivars and commercial hybrids. This study emphasizes the importance of utilizing molecular markers for assessing genetic diversity, which is crucial for hybrid development. Similarly, Tomkowiak *et al.* (2022) employed DArTseq technology to link SNP markers related to yield-related traits in maize, highlighting the acceleration of yield gains through molecular marker assisted breeding.

Also, the identification of quantitative trait loci (QTL) offers valuable insights into potential strategies for enhancing crop stress tolerance in the field. Several QTL in relation to drought stress and low soil nitrogen have been mapped and identified by several researchers (Collins *et al.*, 2008; Ribeiro *et al.*, 2018; Sarkar *et al.*, 2023; Raj & Nadarajah, 2023). Recent studies in maize genomics and genotyping have led to the identification of new QTL and GWAS associations for drought tolerance and nitrogen-use traits. Sarkar *et al.* (2023) used a recombinant inbred line (RIL) population evaluated under well-watered and water-deficit environments to detect 27 QTL for morphophysiological and yield traits; notably, they reported a co-localized QTL for stomatal conductance and grain yield on chromosome 6. In a complementary approach, Li *et al.* (2025) conducted a GWAS of agronomic traits, stress tolerance indices, and phenotypic plasticity in a multi-parent maize population, detecting over 300 QTL associations (130 for agronomic traits, 171 for stress indices, and 71 for plasticity), indicating that many loci are stress-specific, and only a few are shared among phenotypic classes. Also, Xing *et al.* (2024) integrated QTL, GWAS and multi-omics data to mine stable candidate loci regulating nitrogen-use efficiency in maize.

The use of phenomics and high-throughput phenotyping techniques allows breeders to rapidly screen and evaluate large populations of maize germplasm for stress tolerance. Adak *et al.* (2023) utilized high-throughput phenotyping techniques to monitor the interaction of 520 segregating recombinant inbred lines in both irrigated and drought stress trials. The studies combined phenomics and genomic data to predict flowering times, and it found improved prediction accuracy when these types of data were used. Additionally, the study identified a candidate gene related to heat stress in maize using temporal phenomics data, revealing a time-dependent association between genotypes and abiotic stresses. Overall, the research highlights the potential of high dimensional phenomics data to predict complex traits across different environments and to uncover time-dependent genotype-stress relationships for developing resilient plants.

The application of modern breeding techniques such as MAS and genomic selection has expedited the breeding process and improved the selection efficiency for abiotic stress tolerance. The use of MAS has led to an increase in genetic advancement compared to traditional phenotypic selection, benefiting various attributes such as crop yield, stress resilience, and other qualitative characteristics. Furthermore, the identification of QTLs provides valuable insights into strategies for improving stress tolerance in crops. The combination and application of modern breeding techniques, including the use of high-throughput phenotyping techniques, offer promising avenues to enhance research and the development of resilient crop varieties.

2.5 The role of molecular markers in assessing genetic diversity

The success of crop improvement relies on effectively identifying and integrating genetic diversity from a wide range of plant genetic sources. These sources include currently cultivated and newly developed cultivars, landraces, wild and closely related cultivars, as well as germplasm collections of elite and mutant plants. Conventional methods of assessing genetic diversity, such as the use of

morphological and biochemical markers, often fail to capture the full extent of genetic variation due to their sensitivity to the environment (Reddy *et al.*, 2018; El-Orabey *et al.*, 2020; Ijeomah *et al.*, 2020). The emergence of molecular marker technologies has reformed genetic diversity studies by providing high precision and reproducibility in genetic differentiation and relatedness assessments. Hasan *et al.* (2021) highlighted that molecular marker-assisted selection has significantly reduced the time required to develop new crop varieties. In maize, Shu *et al.* (2021) used SNPs and genotyping by sequencing (GBS) to analyze genetic variation and population structure of summer maize germplasm, demonstrating the effectiveness of molecular markers in reducing the costs associated with genome sequencing and genotyping, which are critical for germplasm studies and molecular breeding.

Molecular markers, such as simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP), single nucleotide polymorphism (SNP), random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR), are essential and have been extensively used in modern plant breeding and genetics. Among these, ISSR markers allow for the analysis of genetic variation at the molecular level and are advantageous due to their high polymorphism, easy to develop and comparatively inexpensive (Gemmill & Grierson, 2021). ISSR markers can be utilized universally across plant phylogenetic diversity with a basic technique that can be modified with options for broad implementation for a broad range of projects and budgets (Gemmill & Grierson, 2021).

The application of ISSR markers has been validated in various plant species (Soliman *et al.*, 2014; Yousefiazarkhanian *et al.*, 2016; Arya *et al.*, 2022; Kumawat *et al.*, 2023;). In maize, ISSR markers have been utilized to successfully assess genetic diversity and phylogenetic relationships among cultivars. Filiz *et al.* (2024) reported 180 loci, of which 161 were polymorphic, across 40 genotypes

while Soliman *et al.* (2021) similarly detected 161 polymorphic markers from 13 ISSR primers across 40 maize accessions, indicating a high level of polymorphism. Comparing SSR and ISSR markers, Kausar (2022) highlighted significant genetic diversity among maize genotypes using ISSR markers. Similarly, Al-naggar *et al.* (2022) assessed genetic diversity among maize inbred lines using ISSR markers, revealing significant relationships with hybrid performance.

Overall, ISSR marker applications overcome most of the practical limitations associated with SSR, AFLP and RAPD analysis (Ebrahimi *et al.*, 2015; Padmakar *et al.*, 2015), making them a reliable cost-effective tool for crop improvement programmes where understanding genetic variation is critical for selecting desirable agronomic traits. The main advantage of ISSRs is that they do not require prior knowledge of DNA sequences to design primer sequences, also, the application of polymerase chain reaction (PCR) makes it possible for a minimal amount of DNA to be used in ISSR analysis (Amiteye, 2021).

2.6 Secondary traits for abiotic stress tolerance in maize

Achieving high and stable yields under abiotic stress continues to be a crucial objective in plant breeding however direct selection for yield is ineffective due to its low heritability. Selection under ambient field stress conditions is also inefficient due to the substantial natural variability present in field environments (Božović *et al.*, 2022; Ige *et al.*, 2023). Naggar *et al.* (2020) emphasized that while genetic variability exists among maize hybrids under drought and low nitrogen conditions, yield selection is constrained by genotype x environment interactions. This necessitates a more nuanced approach to breeding, incorporating multiple traits rather than focusing solely on yield.

To improve genetic gain when selecting under stress, it is important selection is done using secondary traits. This is because under stress conditions secondary traits can be used to indirectly

select for stress tolerance related traits with higher heritability and genetic correlation, which is more effective than direct selection for yield (Ziyomo & Bernardo, 2013; Rahman *et al.*, 2021). Selection using secondary traits is advantageous when the secondary trait is highly heritable, highly genetically correlated with the target trait, and it is inexpensive to measure relative to the target trait (Rutkoski *et al.*, 2016). In practice, breeders improving maize for stress tolerance, including drought and low soil fertility, increasingly rely on secondary traits and selection indices to identify superior genotypes under stress conditions (Tigchelaar *et al.*, 2018; Nelimor *et al.*, 2020; Badu-Apraku *et al.*, 2023;).

Several studies have validated the effectiveness of secondary traits in maize breeding. Iseghohi *et al.* (2022) found significant correlation between anthesis-silking interval (ASI) and grain yield under drought stress condition. Their study indicated that a shorter ASI is associated with better grain yield performance. Oyekunle *et al.* (2019) also reported significant correlations between grain yield and secondary traits such as number of ears per plant (EPP) and plant aspect under both drought and well-watered environments. Their results suggest that these traits can be effectively used in selection indices to improve grain yield in maize breeding programmes (Oyekunle *et al.*, 2019).

Also, under drought and low-nitrogen conditions, selection based on secondary traits improves the accuracy of identifying and developing stress-tolerant genotypes. Naggar *et al.*, (2020), using a split-split-plot design across irrigation and nitrogen levels identified high 100-kernel weight (100-KW), number of ears per plant (EPP), number of kernels per row (KPR), and short anthesis-silking interval (ASI) as the best secondary traits for low-N tolerance. For tolerance to drought, high EPP, 100-KW, plant height (PH) and short ASI were identified as the best secondary traits, while high 100-KW, EPP, PH, and short ASI, were identified as the best secondary traits for tolerance to

drought combined with low N. The study further recommended that future research breeding programmes under low N and/or drought should focus on incorporating EPP, KPR, 100-KW, PH and ASI with grain yield when selecting for ideal maize genotypes. Other studies also identified secondary traits, such as plant aspect, plant and ear heights, stay green characteristic, and ear aspect, as reliable traits for selection under drought and low N environments (Badu-Apraku *et al.*, 2012; Talabi *et al.*, 2017).

2.7 The utilization of Heterosis and Hybrid development in Crop Improvement

Heterosis, often referred to as hybrid vigour, refers to the phenomenon where offspring of two different lines or varieties exhibit superior performance compared to their parents. This phenomenon has been attributed to genetic factors such as dominance and over-dominance based on individual gene loci, as well as epistasis based on the interactions among genes (Baranwal *et al.*, 2012; Labroo *et al.*, 2021). In plants, heterosis is further expounded by the interaction among multiple loci, depending on hybrids and traits (Schnable & Springer, 2013), as exhibited in flowering related traits (H. Li *et al.*, 2017), and resistance to abiotic and biotic stresses (Miller *et al.*, 2015). For breeders, achieving desirable heterosis remains central to the development of improved crop varieties that have better yields, better disease resistance, and other improved traits compared to the parental lines.

Harnessing heterosis is of great importance in crop development with maize being one of the most successful examples. Maize was one of the earliest crops to benefit from the power of heterosis by breeding first filial (F_1) hybrids exhibiting superior vigour for plant growth and grain yield (Xiao *et al.*, 2021). However, *per se* parental performance is not a clear indication of hybrid performance as excellent hybrids are not necessarily derived from elite parents (Yu *et al.*, 2020). Combining ability is an effective tool for determining the best parental lines for use in hybrid development,

whether to exploit heterosis or to accumulate productive genes (Aswin *et al.*, 2020; Chandra, 2022). Both general combining ability (GCA) and specific combining ability (SCA) provide insight into the contribution of additive and non-additive gene effects, respectively, and are critical for hybrid development.

To maximize heterosis for hybrid development, the available germplasm must be placed into distinct heterotic groups. The classification of inbred lines into distinct heterotic groups is critical for optimizing their utilization in the development of high yielding hybrids and synthetic varieties. Heterotic groups are made up of related or unrelated genotypes that develop superior genotypes when crossed with genotypes from another heterotic group. Classification of germplasm into separate heterotic groups has been a powerful tool used by plant breeders to identify ideal parents for hybrid development using genetic and phenotypic performance. The success of a hybrid breeding programme depends on the availability and effective utilization of heterotic groups and patterns to maximize grain-yield heterosis (Begna, 2021) thereby preventing the development of non-useful or poor performing hybrids.

The use of SCA effects of grain yield to classify maize inbred lines into heterotic groups has been used by several researchers (Shaibu *et al.*, 2021; Zebire *et al.*, 2022; Kumar *et al.*, 2022; Maazou *et al.*, 2023) however, SCA effects of grain yield are often influenced by the interaction between two inbred lines and the environment (Osuman *et al.*, 2022; Mebratu *et al.*, 2024;). Studies by (Banoth *et al.*, 2021) discussed how the classification of inbred lines into heterotic groups based on SCA effects can vary due to environmental interactions, leading to different group assignments in various studies.

Alternatively, the heterotic group's specific and general combining ability (HSGCA) method, proposed by Fan *et al.* (2008) integrates both SCA and GCA effects of grain yield to assign inbred lines into heterotic groups. However, assigning inbred lines based on grain yield is challenging because it is a polygenic trait, influenced by other traits and has low heritability under environmental stress. To address this, Badu-Apraku *et al.* (2013) introduced the heterotic grouping based on the GCA of multiple traits (HGCAMT) method. This approach considers multiple traits associated with grain yield of inbred lines with significant GCA effects across diverse environments, making classification more predictable and reliable. Since GCA measures additive gene effects, HGCAMT is particularly effective for breeding under stress environments. Additionally, molecular markers have also been used to complement phenotypic approaches, offering insights into genetic similarity or genetic distance for heterotic grouping of inbred lines (Kumar *et al.*, 2022).

Studies have been conducted to establish heterotic patterns in tropical maize germplasm (Badu-Apraku *et al.*, 2016; Oyetunde *et al.*, 2020; Osuman *et al.*, 2022). In a study to identify combining abilities and heterotic patterns of thirty (30) tropical maize inbred lines under optimal and Striga-infested environments, Adu *et al.* (2022), using the HGCAMT method identified three distinct groups under optimal environments and across two research conditions, while four clusters were observed under Striga-infested environments. Belay (2022), applying the HSGCA method in a line x tester design, classified fifteen (15) inbred lines into two heterotic groups with hybrid combinations such as L5 x T1 and L7 x T2, derived from distinct heterotic groups, exhibiting superior grain yield. These findings demonstrate the importance of integrating heterotic grouping strategies with combining ability analysis to guide efficient hybrid development.

2.8 Enhancing Genetic Gain Through Multi-location trials: Evaluating Maize Stress Tolerance under Drought and Low N conditions in Agricultural Research

2.8.1 Genotype x Environment Interaction (GEI)

Genotypic expression of a phenotype is strongly influenced by the environment, particularly when genotypes respond differently under varying conditions. This is further explained when a genotype performs optimally in one environment but performs poorly in another. A key component of understanding the performance of maize hybrids across various environments is the role of genotype by environment interactions (GEI) as it significantly influences breeding strategies and the selection of superior genotypes. Multi-location trials (MLTs) are essential in understanding these interactions, providing a holistic approach to assess the performance of crops across diverse environments. The need for reliable and region-specific performance has driven researchers, including plant breeders, to adopt MLTs, enabling them to account for variations in soil types, climate patterns, and other factors that significantly impact agricultural productivity (Ehemba *et al.*, 2019; Tolley *et al.*, 2023; Anthony *et al.*, 2024).

Studies have shown that GEI can lead to variations in the ranking of maize genotypes across different environments, hence the need for costly multi-environment trials (METs) to optimize hybrid development and placement (Ohunakin *et al.*, 2021; Tolley *et al.*, 2023). This variability underscores the importance of conducting trials in multiple environments to ensure that selected hybrids exhibit consistent performance (Ruswandi *et al.*, 2021a; Wicaksana *et al.*, 2022). These trials enable breeders to categorize environments into mega-environments, facilitating the selection of genotypes that are best suited for specific conditions (Apala Mafouasson *et al.*, 2018; Katsenios *et al.*, 2021). In the evaluation of 110 F_1 maize hybrids developed via a complete diallel cross, under both low- and optimum-nitrogen environments in six environments, Dosho *et al.*, 2022, identified significant yield variations of the hybrids which was attributed to the genotypes,

environments, and GEI. Similarly, Badu-Apraku *et al.* (2023) identified three mega-environments across 14 stress sites and identified superior hybrids with broad and specific adaptability.

The complexity of GEI poses a challenge in predicting the performance of selected genotypes under varying conditions. However, the use of statistical models such as Additive Main Effects and Multiplicative Interaction (AMMI) and Genotype main effect plus Genotype-by-Environment interaction (GGE) biplot analysis have been effectively used to assess the stability and adaptability of maize hybrids across diverse environments (Abid & Zahid, 2018; Gungor *et al.*, 2022; Lydia Pramitha *et al.*, 2022; Ruswandi *et al.*, 2022; Karjagi *et al.*, 2023).

The use of GGE biplots has been instrumental in assessing the stability and adaptability of maize hybrids, allowing breeders to identify those that perform well across multiple environments (Božović *et al.*, 2018; Ruswandi *et al.*, 2022). This is particularly relevant in regions with diverse agro-ecologies, where environmental factors such as soil type, moisture availability, and disease pressure can vary significantly (Ohunakin *et al.*, 2021; Keno *et al.*, 2023). Integrating genomic data with environmental characterization is increasingly being recognized as a valuable approach to enhance the understanding of GEI and improve the efficiency of maize breeding programmes (Rogers *et al.*, 2021; Li *et al.*, 2022).

2.8.2 Yield Stability

The primary goal of plant breeding is to develop high-yielding crop varieties with wide adaptability and stability across diverse environments. Conversely, GEI also gives breeders the opportunity to identify and select genotypes that are adapted or perform well at specific locations or across environments. However, yield stability is particularly challenging to achieve because

yield is a polygenic quantitative trait with low heritability and strongly influenced by environmental variation.

The analysis of MLTs data often results in GEI effects, making result interpretation difficult and reducing the efficiency of selecting superior genotypes. To overcome these challenges, the two widely used biplot models are AMMI biplot = the additive main effects and multiplicative interaction and GGE biplot = genotype + genotype x environment (Khan *et al.*, 2021). Both provide multivariate analyses that integrate genotype performance with environmental interactions. Additionally, the Best Linear Unbiased Prediction (BLUP), a statistical model widely used in MLTs data analysis, serves as an optimal selection procedure by maximizing selection accuracy and integrating multiple sources of information. BLUP addresses data imbalances, considers the genetic relationships among plants evaluated, and accounts for the coincidence between the selection and recombination units, making it a powerful tool in plant breeding (Santos & Marza, 2020).

Biplots graphically show the interrelationships between genotypes (G), environments (E), and GEIs, as well as to demonstrate interaction patterns thereby helping breeders identify genotypes that are either broadly adapted or specifically suited to particular environments (Oladosu *et al.*, 2017). The AMMI biplot model integrates both additive and multiplicative effects, allowing for a comprehensive analysis of how different genotypes perform under varying environmental conditions (Kumar *et al.*, 2020; Rao *et al.*, 2022). The most prominent feature of AMMI analysis is its visually informative biplot presentation, which effectively illustrates both the primary and interactive influences of genotype and environment. Despite this feature, the AMMI biplot lacks the key characteristic of a true biplot, namely the inner-product property, and does not display the discriminating ability and representativeness view that are essential for effectively evaluating test

environments (Olivoto *et al.*, 2019; Liu *et al.*, 2022). Nevertheless, the effectiveness of the AMMI procedure has been clearly demonstrated by various researchers in various crops such as maize, wheat, bambara groundnut and rice (Badu-Apraku *et al.*, 2012; Naroui Rad *et al.*, 2013; Akter & Hassan M, 2014; Khan *et al.*, 2021).

The GGE biplot has several advantages over the AMMI biplot, as it possesses the inner-product property, displays both the mean performance and stability of each genotype, and illustrates the relative performance of genotypes across varying environments. It is a valuable statistical tool for developing phenotypically stable and superior cultivars, identifying stable genotypes across several environments, and achieving yield stability (Khan *et al.*, 2021). Additionally, the GGE biplot has been widely utilized in crop variety trials to identify superior genotypes across diverse environments, delineate mega-environments for targeted genotype recommendations, and assess both the yield and stability of genotypes (Ding *et al.*, 2008; Rakshit *et al.*, 2012; Olanrewaju *et al.*, 2021).

The AMMI model is frequently employed alongside the GGE biplot to identify mega-environments (MEs) as well as best performing genotypes in each environment. The BLUP method, which estimates the mean yield of genotypes in mixed models with high efficiency, has also been used to effectively to analyze METs (Santos & Marza, 2020; A.v.v. *et al.*, 2021; Gela *et al.*, 2022; Taleghani *et al.*, 2023).

To utilize the advantages of both AMMI and BLUP methodologies, an index known as the Weighted Average Absolute Scores of BLUPs (WAASB) has been developed, which is a combination of AMMI and BLUP (Olivoto *et al.*, 2019). In the identification and introduction of new crop varieties, breeders simultaneously evaluate for both stability and yield attributes, in

addition to mitigate GEI while selecting genotypes with high yield potential; therefore, considering the WAASB index and yield (Y), the WAASBY index was introduced, which allows for the simultaneous consideration of stability and yield characteristics (Olivoto *et al.*, 2019).

The utilization of advanced statistical tools such as AMMI and GGE biplots, along with BLUP methodology, has emerged as indispensable strategies for overcoming these challenges in MLTs data analysis. While AMMI biplot offers insightful visualization of GEI patterns, it may lack certain essential characteristics, such as the inner-product property. Conversely, the GGE biplot, with its inherent advantages including the inner-product property, provides a comprehensive assessment of genotype performance and stability across environments. The integration of AMMI and BLUP methodologies through the WAASB index signifies a significant advancement in enhancing the efficiency and accuracy of cultivar evaluation. Also, the introduction of the WAASBY index underscores the importance of considering both stability and yield attributes in cultivar selection, thereby addressing the multifaceted challenges posed by GEI. By leveraging these innovative approaches, breeders can navigate the complexities of GEI more effectively, ultimately contributing to the development of resilient crop varieties that meet the evolving needs of agricultural systems.

In addition to the mentioned methods, classical methods such as the Finlay and Wilkinson model and the Eberhart and Russell model are particularly important for their contributions to understanding the stability and adaptability of genotypes across different environments. The Finlay and Wilkinson model, introduced in 1963, posits that genotypic performance can be predicted based on its average yield across environments and has been effectively used to identify genotypes that are stable across a range of conditions, making it a foundational tool in stability analysis (Kassa *et al.*, 2006; Naidu *et al.*, 2017). However, while this model provides valuable insights, it

does not account for the multiplicative nature of GEI, which can limit its applicability in complex scenarios (Scapim *et al.*, 2000). In contrast, the Eberhart and Russel method, developed in 1996, allows for a more nuanced understanding of stability, as it distinguishes between genotypes that perform consistently across environments and those that exhibit variable responses (Kumar & Dhillon, 2020; Mohammed & Mohammed, 2020). This model is particularly useful for identifying high-yielding genotypes that maintain performance stability, making it a widely adopted method in agricultural research (Sowmya *et al.*, 2018).

Overall, the pursuit of yield stability requires integrating diverse statistical tools and indices to effectively interpret GEI. By leveraging on these models, breeders can identify genotypes with broad adaptability or specific adaptation, ultimately contributing to the development of stress-resilient and high yielding crop varieties for different agro-ecologies.

CHAPTER THREE

3.0 GENETIC DIVERSITY OF MAIZE INBRED LINES UNDER VARYING ENVIRONMENTS

3.1 Introduction

On most farmer fields, apart from drought and low soil fertility, weed competition significantly impacts maize productivity. Due to the high cost of manual weeding and labour shortages, the use of selective herbicides such as nicosulfuron, a sulfonylurea herbicide that inhibits acetolactate synthase (ALS) enzyme activity, has been widely adopted. However, the susceptibility of one of the parental lines used in two high-yielding WACCI varieties, *Aburo Legon* and *Abenfo Aburo,* to nicosulfuron, has impeded the seed production of these varieties thereby limiting its adoption and utilization by farmers in Ghana. Apart from the observed susceptibility of the WACCI released hybrids to nicosulfuron, there have been other studies that have shown varying tolerance levels of maize varieties to nicosulfuron (Cavalieri *et al.*, 2008; Cavalieri *et al.*, 2010).

In the face of climate change and continuous change in cultivation conditions, plant breeders are constantly faced with the increasing demand for resilient varieties.Genetic diversity is an important resource for breeders to develop new varieties with improved traits such as high yields, biotic and abiotic stress tolerance and improved nutrition. Phenotypic diversity which is driven by genetic variation is crucial for ensuring the adaptability and performance of crop varieties across varying environments. Depending on the environmental conditions, in crops such as maize, phenotypic traits such as grain yield, days to anthesis and silking, and plant height vary, making it crucial to assess genetic variation for breeding programmes.

The success of crop improvement lies in efficiently identifying and incorporating genetic diversity from various plant genetic sources including currently cultivated cultivars, newly developed

cultivars, landraces, wild and near relatives of cultivated cultivars, and germplasm collections with elite and/or mutant plants (Swarup *et al.*, 2021). The maize breeding programme at the West Africa Centre for Crop Improvement (WACCI) leverages genetic diversity from collected and developed parental lines to develop hybrids that would contribute to improved maize yields in Ghana. However, the performance of newly developed maize inbred lines across varying environments, remains inadequately understood as there is limited information. Evaluating genetic diversity and its relationship to plant performance under varying environments is a crucial step for the identification of elite inbred lines with broad adaptability and resilience.

Many types of molecular markers have been employed for the characterization and assessment of genetic diversity and relationships in plant species including maize. These include RFLPs (restriction fragment length polymorphisms), RAPDS (randomly amplified polymorphic DNAs), AFLP (amplified fragment length polymorphisms), SNPs (single-nucleotide polymorphic), SSRs (simple sequence repeats), and ISSRs (inter-simple sequence repeats). RAPDs have low reproducibility, RFLPs are time-consuming and labour intensive, SSRs require the knowledge of the flanking regions for the development of species-specific primers, and AFLPs and SNPs have high cost (Reddy *et al.*, 2002). However, ISSR markers and hypervariable, highly reproducible, fast, affordable, and does not require any prior sequence information of amplified locus (Zietkiwewicz *et al.*, 1994; Reddy *et al.*, 2002). ISSR-PCR detects the level of variation in microsatellite regions and gives multi-locus schemes, which are iterative and polymorphic in plant genomes (Agostini *et al.*, 2008; Yu *et al.*, 2011).

This highlights the importance of including genetic diversity assessments with performance evaluation under varying environments to ensure the identification of elite lines in developing robust hybrids.

The objective of this study were to:

(i) assess the performance of maize inbred lines under optimum, drought and herbicide-treated environments, and

(ii) assess the genetic diversity among selected maize inbred lines using ISSR markers.

3.2 Materials and methods

3.2.1 Description of germplasm

One hundred maize inbred lines, were randomly selected from the germplasm pool at WACCI, comprising 48 white (W) kernel and 52 yellow (Y) kernel maize inbred lines and used in this study (Appendix 1). The inbred lines represented diverse genetic backgrounds sourced from International Institute of Tropical Agriculture (IITA), Institute of Agricultural Research for Development (IRAD) and WACCI. Among these, sixty-seven (67) inbred lines were developed from five (5) base populations using the double haploid (DH) technology, while others included mutant populations generated through ethyl methane sulfonate (EMS) and gamma irradiation (GY) for the development of herbicide-tolerant and fall armyworm resistant lines. The mutant lines were at the M5-M6 generation and designated as EMS or GY lines. The inclusion of these lines was to evaluate their phenotypic response under herbicide and drought stress environments, thereby identifying potential tolerance sources for future breeding.

3.2.2 Experimental site and field layout

The experiments were conducted at the WACCI Research Field in Legon, Accra (5°38' N lat., 00°11' E long.; altitude 97 m above sea level). The site experiences a bimodal rainfall pattern with an annual mean rainfall of 809 mm and temperatures of 23°C and 31°C. The soils at Legon are

classified as belonging to the Adenta series with a sandy clay loam texture in the surface horizon and sandy clay texture in the lower horizon (Eze, 2015).

Three environmental conditions were evaluated:

i. Optimum environment (well-watered and manually weeded);
ii. Herbicide-treated environment (well-watered and stress due to nicosulfuron application for weed control and to identify tolerant inbred lines); and
iii. Drought stress environment (moisture-limited).

The optimum and herbicide-treated experiments was conducted in 2022, during the minor rainy season, using a 10 x 10 lattice design with two replications under each experiment. Each plot across all experiments consisted of single 4 m row plots, with 0.75 m spacing between rows and 0.4 m between plants within a row. Three seeds were planted per hill and thinned to two plants per hill at two weeks after emergence, resulting in a plant population density of 66,666 per hectare.

3.2.2 Optimum and Herbicide-control treatment

Manual weeding was employed for the optimum plots, while herbicide-treated plots were sprayed with *Nicoking,* which has an active ingredient of nicosulfuron, a post-emergence systemic sulfonylurea herbicide used for selective weed control in maize (Brankov *et al.*, 2024; Cinelli *et al.*, 2020). The whole field include the planted maize inbred lines was directly sprayed with the chemical. This was done to primarily evaluate the susceptibility of maize inbred lines to the herbicide sprayed. A 15 litre (L) knapsack sprayer fitted with a flat-fan nozzle was used in the application of the herbicide at the recommended rate of 95 mL per 15 L of water.

Herbicide application was done at two different growth stages of the crop to test the susceptibility of the inbred lines to the herbicide. The first spraying was done at the V3-V5 (early vegetative with 3-5 fully expanded leaves) growth stage of the plant while the second spraying was done at V14-VT (late vegetative to tasseling) growth stage. These stages were selected to assess both early vegetative tolerance and potential recovery or injury persistence during reproductive/tasseling stage.

3.2.3 Drought stress environment

The drought experiment was conducted at the WACCI Research Field, Legon, during the dry season (December 2022 – February 2023) using a subset of 50 maize inbred lines. These lines were selected from the 100 inbred lines previously evaluated under the optimum and herbicide-treated environments based on adequate seed availability and *per se* performance, ensuring representation from both white- and yellow-kernel groups as well as different genetic sources. The experiment used a 5 x 10 lattice design with two replications, however since all 50 entries were included within each replication, the layout functionally operated as a randomized complete block design (RCBD).

To impose reproductive-stage drought stress, watering was withheld at approximately six to seven weeks after planting (6 – 7 WAP), corresponding to the pre-flowering stage, which represents the critical period leading to anthesis and silking. This timing ensured that moisture stress coincided with tassel and silk development which are the stages most sensitive to water deficit.

Irrigation was to be resumed two weeks after silking to support grain filling and physiological maturity, however intermittent rainfall occurred during the stress period, partially mitigating the

intended water deficit hence no irrigation was done on the field. Consequently, the stress experienced by the plants reflected moderate rather than severe drought intensity.

All other agronomic practices, including fertilizer application and pest control, were consistent with those used under the optimum environment, except that no supplementary irrigation was provided during the stress period.

3.2.4 Fertilizer application

All experiments received 60 kg ha^{-1} each of nitrogen (N), phosphorous (P) and potassium (K) applied as compound fertilizer (15-15-15 NPK) at 2 WAP, followed by an additional 30 kg ha^{-1} N as urea at 5 WAP.

3.2.5 Data collection

The following data were collected and recorded on per plot basis:

Plant stand (PLST) = number of plants in a plot soon after thinning.

Days to anthesis (DYA) = number of days from planting to the date when 50% of the plants have tassels shedding pollen.

Days to silking (DYS) = number of days from planting to the date when 50% of the plants have emerged silks.

Anthesis-silking interval (ASI) = the difference between days to 50% silking and 50% anthesis.

Plant height (PLHT) = Average height of plants in centimetres (cm) from the base of the plant to the point of tassel branching.

Ear height (EHT) = Average height in cm from the base of the plant to the node bearing the upper ear.

Plant aspect (PASP) = General score for plant appearance in the plot, considering relative plant and ear heights, uniformity, reactions to insects and diseases, lodging, etc. PASP was rated on a scale of 1 to 5 where 1 = excellent overall phenotypic appeal and 5 = poor overall phenotypic appeal.

Root lodging (RL) = number of plants that fell from the root.

Stalk lodging (SL) = number of plants with broken stalk below the ear or the stalk bending more than 45° from the upright position.

Plants harvested (PHARV) = the total number of plants harvested.

Ears harvested (EHARV) = the total number of ears with at least one fully developed grain harvested.

Number of ears per plant (EPP) = the total number of ears with at least one fully developed grain divided by the number of harvested plants.

Ear aspect (EASP) = assessment of the general appeal of the ears after dehusking on a scale of 1 – 5. The factors considered included ear size, uniformity of size, extent of grain filling, insect and disease damage where 1= excellent with no disease/insect damage, uniform ears and fully filled cobs and 5 = only one or no ears with severe ear insect/disease damage and scanty or no grain filling.

Additional data measured under the herbicide-tolerance environment included:

Crop tolerance (CT) = this is a measure of the level of phytotoxicity of the inbred line to the herbicide applied. This was determined using the European Weed Research Council (EWRC) rating scale for phytotoxicity of 1 – 9 (WSSA, 2002) where:

1 = no effect

2 = very slight effects; some stunting and yellowing just visible.

3 = slight effects, stunting and yellowing effects reversible.

4 = substantial chlorosis and/or stunting; most effects probably reversible.

5 = strong chlorosis/stunting; thinning of stand.

6 = increasing severity of damage.

7 = increasing severity of damage.

8 = increasing severity of damage.

9 = total loss of plant yield.

On the herbicide-treated field, data on plant stand was taken at 7 days and 21 days after the first spraying. Data on plant stand was taken again 21 days after the second spraying was done. Data on crop tolerance was taken at 7 days (CT1) and 21 days (CT2) after the first spraying. These combined measurements provided a reliable index of herbicide tolerance or susceptibility among the tested maize inbred lines.

For stress environments (herbicide-treated and drought), all ears harvested per plot were hand-shelled and grain weight per plot was recorded. Grain yield (GY) was adjusted to 15% moisture and estimated from the shelled grain weight (Badu-Apraku & Akinwale, 2011). This was calculated as follows:

$$GY = Gwt\ x\ \frac{(100 - m)}{85}\ x\ \frac{10000}{(8 \times \emptyset)}$$

where:

GY = grain yield (kg/ha),

Gwt = shelled grain weight per plot (kg),

m = moisture content of grain at harvest,

10,000 = land area per hectare (m^2),

8 = land area per plot,

ϕ = number of hills per plot.

For the optimum environment, where only field ear weights were recorded, grain yield was calculated as follows:

$$GY = fwt \times \frac{(100 - m)}{85} \times \frac{1000}{(8 \times \phi)} \times 0.8$$

where;

fwt = field weight of harvested ears per plot (kg),

0.8 = 80% shelling percentage (I. K. Amegbor *et al.*, 2020a).

3.2.6 Genetic diversity using ISSR markers

Fifty (50) white and yellow kernel maize inbred lines, selected for the drought experiment, were used for the molecular diversity assessment (Table 3.1). Inter simple sequence repeat (ISSR) markers were used to evaluate the genetic diversity and relationships among these inbred lines because of their reproducibility, cost effectiveness and ability to detect polymorphisms across the genome.

Young leaf tissues were collected from 2-week-old seedlings grown in seed trays. For each inbred line, leaf samples were collected from 10 representative plants, placed in paper envelopes, and promptly freeze-dried. Genomic DNA was then extracted from the freeze-dried leaves of each inbred line using a modified cetyltrimethylammonium bromide (CTAB) method following Aboul-Maaty and Oraby (2019) with adjustments based on McGarvey and Kaper (1991).

Approximately 200 mg of leaf tissue was macerated in 1.5 ml micro-centrifuge tubes using sterile plastic pestles. One millilitre (1 mL) of extraction buffer (7% CTAB, 1% polyvinylpyrrolidone, 0·1 m Tris–HCl, 1·4 m NaCl, 20 mm EDTA, pH 8·0), 300 µL 20% sodium dodecyl sulphate and 0·5% mercaptoethanol (v/v) were added and mixed by vortexing. The mixture was incubated at

55°C for 15 min and the homogenates were extracted with phenol:chloroform:isoamyl alcohol (25:24:1, v/v/v).

The aqueous phase was transferred to a fresh micro-centrifuge tubes and incubated with RNase A (10 µg/ml) at 37°C for 20 min to remove RNA contaminants. DNA was then precipitated with 0.7 volume cold isopropanol and incubated on ice for 15 min. The precipitate was collected by centrifugation 10 000 revs/min for 10 minutes, washed once with 70% ethanol, air-dried, and re-suspended in 100 µL Tris-EDTA (TE) (10 mM Tris, 1 mM EDTA) buffer [pH 8.0] and stored at -20°C for further analysis. DNA concentration and purity were assessed using a Nanodrop spectrophotometer and by 1% agarose gel electrophoresis.

A total of 30 ISSR primers, synthesized by Inqaba Biotech, South Africa, were screened for amplification and polymorphism. Out of these, only 10 primers produced clear, reproducible and polymorphic banding profiles and were selected for analysis. PCR amplifications of the extracted maize DNA were performed using ISSR primers in BIO-RAD PTC-220 thermocycler (Dyad MJ Research). PCR reactions (total volume of 25 µl) containing 12.50 µl of OneTaq Quick-Load 2x Master Mix with Standard Buffer, 0.5 µM each of the primer (from a 10 µM stock solution), approximately 5 ng of genomic DNA and nuclease-free water was prepared. A touch-down PCR amplification profile was used with an initial denaturation cycle at 94°C for 3 minutes (min), followed by 20 cycles of denaturation at 94°C for 30 seconds (s), annealing from 60 °C decreasing by 0.5°C per cycle to 50°C for 1 min and extension at 72°C for 30 s; followed by 30 cycles of 94 °C for 20 s, 55°C for 1 min and 72 °C for 30 s, with a final extension at 72 °C for 7 min.

PCR products were separated by electrophoresis on a 1.2% (w/v) agarose gel in 1x TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0), stained with ethidium bromide and run at 80 V for

90 min. The gels were visualized under UV light using a Cole Palmer FLUO-LINK FLX imaging system, and banding patterns were photographed for scoring. Only distinct and reproducible bands were considered for further data analysis.

3.3 Data analyses

3.3.1 Phenotypic data analysis

Analysis of variance (ANOVA) was performed using the general linear mixed model in R to account for both fixed and random effects inherent in the experimental design of the trial (R core team, 2020). Replications and environments were considered as random factors and inbreds as fixed factors. The entry means were adjusted for block effects, in accordance with the lattice design (Cochran and Cox, 1960). The general linear mixed model is expressed mathematically as:

$$Y_{ijk} = \mu + T_i + R_j + B_{k(j)} + \epsilon_{ijk}$$

where:

Y_{ijk} is the observed response for the genotype grown in the environment i, in the block k in replicate j; μ is the overall mean; T_i is the fixed effect of the treatment (genotype); R_j is the effect of the replicate j; $B_{k(j)}$ is the effect of the block k within replicate j and ϵ_{ijk} is the error term.

Data analysis was done separately on plot means for grain yield and other measured traits under each environment using linear mixed model in R to account for both fixed and random effects inherent in the experimental design of the trial. The **'lme4'** package in R was utilized for fitting the model due to its robustness and flexibility in handling complex random effect structures.

Table 3. 1: Pedigree of the fifty maize inbred lines used in the study.

CODE	GENOTYPE	COLOUR	CODE	GENOTYPE	COLOUR
G1	DHL 99 POP 2	WHITE	G26	M131	WHITE
G2	1368	WHITE	G27	1368-150GY(89)	WHITE
G3	DHL 106 POP 5	WHITE	G28	DHL 184 POP 2	WHITE
G4	DHL 130 POP 3	YELLOW	G29	DHL 44 POP 4	WHITE
G5	HP020-4/4/1/6	YELLOW	G30	HP020-5/1(B)/1/7	YELLOW
G6	DHL 31 POP 1	YELLOW	G31	TZIL 25 (43A) 0.4EMS	WHITE
G7	HP020-4/1(A)/1/1	YELLOW	G32	DHL 145 POP 2	WHITE
G8	DHL 46 POP 1	YELLOW	G33	DHL 45 POP 4	WHITE
G9	EXP 124	WHITE	G34	1368-250GY (21)B	WHITE
G10	HP020-6/2(9)/9/6	YELLOW	G35	DHL 142 POP 1	YELLOW
G11	DHL 107 POP 5	WHITE	G36	LMI 135	YELLOW
G12	1368-150GY (119)S	WHITE	G37	LMI 102	YELLOW
G13	TZIL 25 (60E) 0.4EMS	WHITE	G38	DHL 53 POP 4	WHITE
G14	TZIL 25 (35G) 0.4EMS	WHITE	G39	DHL 139 POP 1	YELLOW
G15	LMI 140	YELLOW	G40	DHL 65 POP 5	WHITE
G16	LMI 141	YELLOW	G41	LMI 144	YELLOW
G17	DHL 166 POP 2	WHITE	G42	DHL 18 POP 1	YELLOW
G18	DHL 101 POP 5	WHITE	G43	DHL 122 POP 1	YELLOW
G19	DHL 186 POP 2	WHITE	G44	HP020-5/3/2/3	YELLOW
G20	HP020-1/3(10)/1-12/9	YELLOW	G45	TZIL 25	WHITE
G21	DHL 223 POP 2	WHITE	G46	HP020-5/2(A)/1/7	YELLOW
G22	HP020-4/3/1-5/1	YELLOW	G47	TZIL 25 (40C) 0.4EMS	WHITE
G23	9006	WHITE	G48	TZMI 760	WHITE
G24	DHL 224 POP 2	WHITE	G49	C316-7	WHITE
G25	DHL 194 POP 1	YELLOW	G50	DHL 71 POP 1	YELLOW

Variance estimates were used to compute the broad sense heritability for each trait as follows:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_e^2}{r}}$$

where:

H^2 is broad sense heritability, σ^2_g is the genotypic variance, σ^2_e is the residual variance and r is the number of replications in each environment .(Beavis *et al.*, 2023).

The Best Linear Unbiased Predictions (BLUPs) for all measured traits under each environment was calculated with their corresponding confidence interval to assess the response of the genotypes under the environment.

3.3.2 Stress tolerance indices

Stress tolerance indices were calculated using the following formulae:

(i) Stress intensity (SI) = $1 - (\frac{Ys}{Yp})$……..(Xavier Mhike, 2012)

(ii) Yield stability index (YSI) = $\frac{Ysi}{Ypi}$…… (Lin *et al.*, 1986)

(iii) Stress susceptibility index (SSI) = $\frac{1-YSI}{SI}$ …… (Fischer and Maurer, 1978)

where:

Ysi = yield of genotype under stress condition

Ypi = yield of genotype under optimum condition

Ys = total mean yield under stress condition

Yp = total mean yield under optimum condition

3.3.3 Genetic Correlation Analysis

Phenotypic and genotypic correlations among measured traits were estimated to determine the degree of association between grain yield and related agronomic or physiological traits under each environment (herbicide-control, optimum, and drought).

Genetic correlation coefficients (r_g) were computed using the formula:

$$r_g = \frac{Cov_{XY}}{\sqrt{V_X \times V_Y}}$$

where:

Cov_{XY} = genetic covariance between traits X and Y,

V_x and V_y = genetic variances of traits X and Y, respectively.

Phenotypic correlations (r_p) were estimated similarly using phenotypic covariance and variance components.

Variance and covariance components were derived from the **mixed linear model** fitted using the *lme4* package in **R (version 4.3.1)**. The model was specified as:

$$Y_{ijk} = \mu + G_i + E_j + (GE)_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} is the observed trait value, μ is the overall mean, G_i is the random effect of genotype i, E_j is the fixed effect of environment j, $(GE)_{ij}$ is the genotype-by-environment interaction, and ε_{ijk} is the residual error term.

The correlation matrices were visualized using the **"corrplot"** and **"PerformanceAnalytics"** packages in R, and heatmaps were generated to display the magnitude and direction of associations among traits under each environment.

3.3.4 Genotypic data analysis

For the genotypic data analysis, ISSR banding patterns were scored manually based on the presence (1) or absence (0) of DNA bands across all genotypes generating a binary matrix. A pairwise genetic similarity matrix was calculated based on Jaccard's coefficient, which considers the proportion of shared bands between any two genotypes. The similarity index (J) between individuals *i* and *j* was calculated as :

$$J_{ij} = \frac{a}{a+b+c}$$

where a = number of bands shared by both genotypes,

b = number of bands present in *i* but absent in *j*, and

c = number of bands present in *j* but absent in *i*. (Kosman & Leonard, 2005)

Cluster analysis was performed based on the similarity matrix using the unweighted pair-group method of arithmetic means (UPGMA) algorithm to generate a dendrogram showing the genetic relationships among the inbred lines. The clustering procedure followed the approach as suggested by Randi *et al.* (1989).

Descriptive marker statistics including minor allele frequency, allele number, gene diversity, heterozygosity and polymorphism information content were computed using PowerMarker, version 3.25 (Liu and Muse, 2005). The polymorphism information content (PIC) value of each primer was determined using the formula by Ghislain *et al.* (1999):

$$PIC = 1 - [(p)^2 + (q)^2]$$

in which p denotes the frequency of the allele bands present, and q denotes the frequency of the allele band absent across all genotypes.

In this analysis, each distinct and reproducible band was treated as a single dominant locus with two possible allelic states: presence (1) and absence (0). Thus, p corresponds to the proportion of individuals showing the band (dominant allele), while $q = (1 - p)$ represents those lacking it.

Since ISSR markers are dominant, heterozygosity cannot be directly inferred hence the PIC value indicates the discriminatory power of each marker, reflecting the informativeness of dominant loci rather than co-dominant allelic diversity. Higher PIC values reflecting greater polymorphism and informativeness.

3.5 Results

3.5.1 Phenotypic response of maize inbred lines in response under herbicide and optimal environments

Application of the herbicide, *Nicoking*, resulted in significant reduction in grain yield and growth performance among the inbred lines, highlighting differential tolerance responses. Under herbicide stress, grain yield ranged from 0 to 1282.7 kg ha^{-1} with a mean grain yield of 639.84 kg ha^{-1}, representing approximately one-third of the yield obtained under optimal (manual weeding) conditions, 1921.85 kg ha^{-1}. This decline underscores the phytotoxic effects of herbicide exposure. However, inbred lines such as C316-7, LMI 140, DHL 258 POP 2 and DHL 145 POP 2, maintained yields above the mean with relatively low crop tolerance scores (CT ≤ 4), suggesting inherent tolerance to nicosulfuron (Table 3.2). Notably, DHL 99 POP 2 produced yield above the population mean despite a high crop tolerance score of 6 at 21 days after spraying (DAS).This

suggests that while the genotype exhibited visible herbicide injury symptoms, it possessed a compensatory growth response that enabled recovery and grain production.

Crop tolerance to the herbicide, an indication of the plant phenotypic response, assessed at both 7 DAS and 21 DAS varied from 1 to 9, reflecting genotypic variation in physiological response. The anthesis-silking interval ranged from -1 to 5 days, while plant and ear height varied from 0 (indicating total plant death) to 174.6 cm, and 0 to 63.7 cm, respectively. Plant mortality increased sharply between 7 DAS and 21 DASS, indicating cumulative phytotoxic effects of nicosulfuron. Early mortality, 7 DAS, ranged from 0 to 45%, while late mortality, 21 DAS to 21 DASS, reached 100% in several highly susceptible genotypes (Figure 3.1). Inbred lines such as, C316-7 LMI 140, DHL 145 POP 2 and DHL 184 POP 2 maintained minimal mortality of below 20% throughout all assessment intervals, consistent with their low crop tolerance (Table 3.2) and higher yield retention under stress. Conversely, DHL 179 POP 3, DHL 86 POP 1 and TZIL 25 recorded complete mortality after 21 days after the first spraying, validating their classification as highly susceptible. DHL 99 POP 2 and DHL 35 POP 1 recorded increased mortality after 21 DASS.

Under optimum conditions, where weeds were manually controlled, grain yield ranged from 3989.53 kg ha^{-1} for HP020-5/2(A)/1/7 to 240.14 kg ha^{-1} for CLRCYQ 40, with an average yield of 1921.85 kg ha^{-1} (Table 3.3). This performance represents an average grain yield increase of 200% relative to the herbicide-treated environment. The anthesis-silking interval (ASI) ranged from -3 to 5 days, and plant height varied between 67.6 cm to 159.4 cm, while ear height ranged from 24.1 cm to 69.0 cm. The highest-yielding inbred lines under optimum conditions included HP020-5/2(A)/1/7, TZIL 25 (40C) 0.4 EMS, and HP020-4/3-1-5/1, each exceeding the mean yield by over 70%.

Table 3. 2: Performance of the best 20 and worst 10 inbred lines, including 2 checks evaluated under herbicide-control environment in Legon, 2022.

Inbred	Grain yield (kg/ha)	Crop tolerance (7DAS)	Crop tolerance (21 DAS)	Anthesis-silking interval	Plant aspect	Plant height (cm)	Ear height (cm)	Ear aspect	Ears per plant
C316-7	1282.7	4	3	-1	2	114.6	48.8	2	1
DHL 99 POP 2	1226.5	3	6	0	3	94.1	46.6	3	1
LMI 140	1210.1	3	4	2	3	105.9	44.2	3	1
DHL 258 POP 2	1204.9	2	2	-2	3	124	52.5	2	1
DHL 145 POP 2	1168.5	4	4	2	3	144.6	37.2	2	2
DHL 184 POP 2	1094.2	2	4	3	4	108.9	38.6	3	1
TZIL 25 (43A) 0.4 EMS	1080.5	4	3	3	2	126	63.7	3	1
DHL 130 POP 3	1079.3	5	6	1	4	61.1	23.4	5	1
TZIL 25 (60E) 0.4 EMS	1073.9	5	4	0	2	174.6	56.8	2	1
DHL 142 POP 1	1056.3	2	2	3	2	96	45.8	3	2
TZIL 25 (35G) 0.4EMS	1038.8	4	4	2	3	115.5	53.8	3	1
1368-250GY (21)B	949.2	1	2	3	2	130.1	60.9	3	1
DHL 65 POP 5	947.8	2	2	5	5	90.9	27.7	3	2
LMI 135	942.4	3	4	1	3	115.5	53.1	3	2
1368-150GY (119)S	802.6	2	3	1	2	121.6	52.3	3	1
DHL 186 POP 2	771.3	4	2	3	2	146.6	39.9	4	1
1368-150GY(89)	763.7	3	4	-1	3	102.1	39.5	3	1
DHL 148 POP 5	735.1	2	3	-1	3	109	46.8	2	2
HP020-5/2(A)/1/7	716.5	3	3	1	4	119.3	56.3	4	1
DHL 65 POP 3	672.4	4	3	2	2	109.6	43.7	2	1
*1368	419.9	3	4	1	3	100.4	41.1	3	1
DHL 194 POP 1	0	7	9	0	5	0	0	9	0
DHL 35 POP 1	0	4	7	3	5	28.75	10.65	7	1
DHL 86 POP 1	0	9	9	0	5	0	0	9	0
DHL 89 POP 2	0	2	8	2	5	43.5	11	9	0
HP020-2/5(9)/1/6	0	4	9	0	5	0	0	9	0
HP020-4/1(B)/1/1	0	2	4	0	5	49.8	33.5	7	2
HP020-5/4/6/2	0	7	9	1	5	17.5	3.5	9	0
HP020-6/1(9)/1-7/2	0	5	9	0	5	0	0	9	0
HP020-6/2(9)/9/6	0	6	6	-3	5	31.4	13.4	7	1
*TZIL 25	0	5	9	0	5	0	0	9	0
Mean	639.84	4	5	1	3	80	32.4	5	1
Minimum	0	1	2	-1	2	0	0	2	0
Maximum	1282.7	9	9	5	5	174.6	63.7	9	2
S.E	91	0	0	0	0	10	4	1	0

**Checks – 1368 (herbicide tolerant), TZIL 25 (susceptible)*

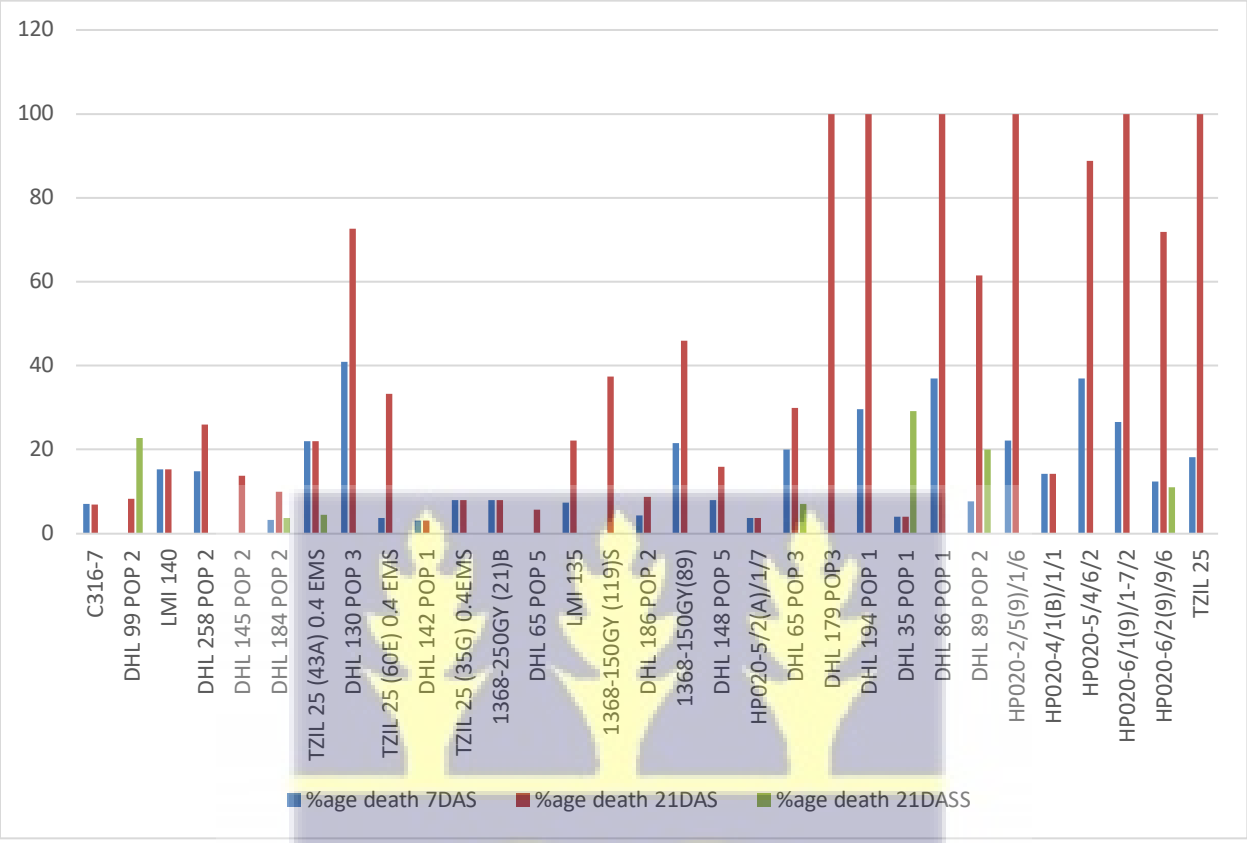

Figure 3. 1: Plant mortality of 20 best and 10 worst performing inbred lines under herbicide-control environment at 7 days after spraying (DAS), 21 DAS and 21 days after second spraying (DASS).

Table 3. 3: Performance of the best 20 and worst 10 inbred lines evaluated under manual weeding (OPT) environment in Legon, 2022.

Inbred	Grain yield (kg/ha)	Days to anthesis	Days to silking	Anthesis-silking	Plant height (cm)	Ear height (cm)	Plant aspect	Ear aspect	Ears per plant
HP020-5/2(A)/1/7	3989.53	62	62	0	138.8	69	2	2	1
TZIL 25 (40C) 0.4 EMS	3484.62	64	65	1	141.6	66.9	2	2	1
HP020-4/3/1-5/1	3370.75	67	69	2	110.5	46	3	2	1
LMI 135	3308.25	64	65	1	141	65.7	2	3	1
HP020-5/1(B)/1/7	3243.09	71	74	3	136	50.6	3	3	1
DHL 122 POP 1	3064.84	65	67	2	137.2	70.4	2	2	1
HP020-6/2(9)/9/6	2920.66	64	64	0	127.3	49.2	2	3	1
9006	2825.07	64	65	1	115	47.6	3	3	1
LMI 102	2763.37	64	66	2	115.5	52.2	3	2	1
DHL 258 POP 2	2735.25	58	56	-2	147.3	65.9	2	3	1
C316-7	2610.63	67	69	3	137.1	65.3	2	2	1
HP020-4/1(A)1/1	2518.48	68	66	-2	135.3	57.6	2	1	1
TZIL 25 (43A) 0.4 EMS	2424.75	64	66	2	132.4	67.1	2	3	1
LMI 141	2315.96	65	66	1	130.1	58.4	2	3	1
LMI 144	2306.32	66	67	2	114.3	48.5	3	3	1
DHL 186 POP 2	2297.39	63	64	1	128.6	50.8	2	3	1
DHL 53 POP 4	2256.92	68	65	-3	159.4	74	2	3	1
TZMI 760	2115.41	70	71	1	116.2	58.5	2	3	1
DHL 119 POP 3	2111.72	69	69	0	117.4	58	3	3	1
DHL 18 POP 1	2058.98	60	63	4	109.3	52.4	2	2	1
DHL 169 POP 2	375.87	64	66	2	88.2	32.5	4	4	1
DHL 109 POP 3	354.24	65	68	3	91.8	37.3	2	3	1
DHL 119 POP 1	352.30	66	68	2	155.7	82.3	3	5	1
DHL 14 POP 1 (A)	307.84	71	76	5	75.3	36.9	3	5	1
DHL 148 POP 3	294.85	69	73	4	92	24.1	3	5	1
DHL 44 POP 4	263.18	64	65	2	139.7	64.9	3	4	1
DHL 14 POP 1	256.09	75	77	2	67.6	27.3	4	2	1
DHL 77 POP 2	247.10	65	70	5	118.7	69.2	3	4	2
TZIL 25 (35G) 0.4EMS	241.96	64	68	4	114.5	51.9	5	5	2
CLRCYQ 40	240.14	71	75	4	92.1	33.7	3	5	2
Mean	**1921.85**	**66**	**67**	**2**	**120.9**	**54.5**	**2**	**3**	**1**
Minimum	**240.14**	**62**	**62**	**0**	**67.6**	**24.05**	**2**	**1**	**1**
Maximum	**3989.53**	**75**	**77**	**5**	**139.7**	**74**	**5**	**5**	**2**
Standard error (S.E.)	**228**	**1**	**1**	**0**	**4**	**3**	**0**	**0**	**0**

The analysis of variance (Table 3.4) revealed highly significant genotypic differences ($p < 0.01$ and $p < 0.001$) among the inbred lines for most agronomic traits and physiological traits under both herbicide-treated (HERB) and optimum (manual weeding; OPT) environments. However, days to 50% (DYANTH) and number of ears per plant (EPP) were not significant under herbicide treatment, while EPP also showed no significant variation under optimum conditions. Notable differences were observed in the contribution of replication and block effects, particularly under the herbicide environment, indicating that environmental heterogeneity was more pronounced under the stress environment.

This suggests that spatial variation in soil properties and herbicide distribution contributed to differential plant responses across the field. The lattice design was therefore effective in minimizing random error and improving the precision of genotype comparisons in a non-uniform experimental field.

The combined ANOVA across both environments (Table 3.5) further showed significant genotypic variation ($p < 0.05$ and $p < 0.001$) for all traits except EPP, confirming substantial genetic diversity among the evaluated lines. Significant genotype x environment (G x E) interaction effects were detected for most traits, implying that the performance that the performance of the inbred lines were not consistent across environments. Environmental main effects were also significant for all traits with the exception of DYANTH, ASI, and EPP, highlighting the strong influence of herbicide stress on growth and yield-related traits. Broad sense heritability (H^2) estimates on plot mean basis ranged from 19% (EPP) to 62% (DYSK) under HERB, with grain yield (YLD) showing a heritability estimate of 20% (Table 3.4). Under OPT, H^2 estimates ranged from 15% for EPP to 59% for YLD (Table 3.4).

Supporting these results, the least significant differences (LSD) summary indicated that the magnitude of genotypic variability was higher under herbicide stress than under optimum conditions (Table 3.6). Under the herbicide environment, coefficients of variation (CV) ranged from 4.8% for days to silking (DYSK) to 14.8% for grain yield (YLD), while under optimum conditions, they ranged from from 4.1% (DYANTH) to 12.4% (YLD), reflecting overall acceptable experimental precision. Larger LSD values for YLD (771.9 kg ha^{-1}) and plant height (56.2 cm) under herbicide stress further confirmed increased genotypic variability induced by chemical injury. In contrast, smaller LSD and CV values under optimum conditions suggested that observed variations were primarily genetic rather than environmental.

Table 3. 4: Mean squares of 100 maize inbred lines screened under herbicide-treated (HERB) and manual weeding (OPT) environment at the WACCI Research Field, Legon in 2022.

HERB					
	Source of variation				
Trait	**Gen**	**Rep**	**Rep (Blk)**	**Residual**	**H^2**
YLD	250512**	257890	271216*	133933	20
MORT	1755.3***	6613.9***	1109.9***	357.8	53
CT1	3.79***	4.43	3.29**	1.49	36
CT2	9.36***	32.85**	6.18*	3.02	41
DYANTH	100.13	75.11	104.02	84.52	47
DYSK	145.63***	268.45*	93.96*	45.89	62
ASI	8.84**	11.67	4.68	4.3596	32
PASP	1.76***	0.77	1.16*	0.83711	47
PLHT	1441.52***	3091.27**	2694.58***	337.03	53
EHT	269.28***	8.63	198.19*	109.29	59
EPP	0.32	0.23	0.56	0.57	19
EASP	2.71*	6.67	2.28	1.72	52
Df	99	1	18	81	
	OPT				
	Gen	**Rep**	**Rep (Blk)**	**Residual**	**H^2**
YLD	1426286***	961	527556	325623	59
DYANTH	36.78***	496.12***	16.65*	7.95	46
DYSK	48.41***	680.8***	24.06	9.27	46
ASI	7.62***	14.58**	2.02	2.07	55
PASP	1.07***	1.82*	0.67*	0.37	38
PLHT	707.9***	7734.6***	768.7***	141.8	34
EHT	307.6***	3808.9***	380.3***	66.6	32
EPP	0.32	0.09	0.24	0.23	15
EASP	1.79***	0.08	0.41	0.59	49
Df	99	1	18	81	

Gen, genotypes; Rep, replication; Blk, Block; CT1, crop tolerance at 7 days after spraying (DAS); CT2, crop tolerance at 21 DAS; DYANTH, days to 50% anthesis; DYSK, days to 50%silking; ASI, anthesis-silking interval; PLHT, plant height; EHT, ear height; EASP, ear aspect; YLD, grain yield (kg/ha); Df, degrees of freedom.

*'***', '**', '*', = significance at p ($\propto = 0.001$), and p ($\propto = 0.01$) and p ($\propto = 0.05$) respectively.*

Table 3. 5: Mean squares of 100 maize inbred lines screened across the herbicide-treated (HERB) and manual weeding (OPT) environment at the WACCI Research Field, Legon in 2022.

	SOURCES OF VARIATION					
Trait	**Gen**	**Environment**	**Gen (Environment)**	**Environment (Rep)**	**Environment (Rep*Blk)**	**Residual**
YLD	948945***	105294743***	673153***	65767	668575	267374
DYANTH	87.56***	125.57	43.19	285.62***	191.24*	40.76
DYSK	119.79***	275.09***	65.77***	474.63***	267.51***	28.59
ASI	11.01***	6.48	4.97**	13.12*	9.39*	3.05
PASP	1.71***	16.48***	1.02***	1.19	0.34	0.58
PLHT	1410.6***	26371.3***	654***	5412.9***	3765.4***	491.3
EHT	408***	8546.6***	153.5***	1908.8***	1635.1***	108.6
EPP	0.56	0.26	0.47	0.32	0.58	0.58
EASP	2.06***	39.23***	2.4***	3.35*	1.09	1.14
Df	99	1	99	2	36	162

Gen, genotypes; Rep, replication; Blk, Block DYANTH, days to 50% anthesis; DYSK, days to 50%silking; ASI, anthesis-silking interval; PLHT, plant height; EHT, ear height; EASP, ear aspect; YLD, grain yield (kg/ha); Df, degrees of freedom.

*'***', '**', '*', = significance at p ($\propto$ = 0.001), and p ($\propto$ = 0.01) and p ($\propto$ = 0.05) respectively.*

Table 3. 6: Summary of mean squares, LSD, and coefficients of variation for agronomic and physiological traits of maize inbred lines evaluated under herbicide treatment and optimum (manual weeding) conditions at Legon, 2022.

HERB						
Trait	**Description**	**Mean**	**LSD (5%)**	**CV (%)**	**F-value**	**Significance**
GY	Grain yield (kg ha^{-1})	639.84	771.91	14.8	1.714	**p < 0.01**
DYANTH	Days to anthesis	58.40	4.85	5.2	2.120	**p < 0.01**
DYSK	Days to silking	59.70	4.99	4.8	1.985	**p < 0.01**
ASI	Anthesis–silking interval (days)	1.30	4.15	10.6	1.898	**p < 0.01**
PLHT	Plant height (cm)	103.20	56.24	8.7	4.380	**p < 0.001**
EHT	Ear height (cm)	43.80	25.72	9.5	4.002	**p < 0.001**
EPP	Ears per plant	1.20	1.46	12.3	1.360	*p < 0.05*
PASP	Plant aspect (1–5)	3.20	1.82	13.1	2.651	**p < 0.001**
EASP	Ear aspect (1–5)	2.60	1.93	11.4	2.442	**p < 0.01**
CT1	Crop tolerance (7 DAS)	2.70	1.96	8.9	3.145	**p < 0.001**
CT2	Crop tolerance (21 DAS)	4.50	2.10	9.7	3.488	**p < 0.001**
OPT						
Trait	**Description**	**Mean**	**LSD (5%)**	**CV (%)**	**F-value**	**Significance**
GY	Grain yield (kg ha^{-1})	1921.85	1256.91	12.4	3.544	**p < 0.001**
DYANTH	Days to anthesis	57.10	3.89	4.1	2.015	**p < 0.01**
DYSK	Days to silking	59.00	3.82	4.5	1.941	**p < 0.01**
ASI	Anthesis–silking interval (days)	1.90	3.47	9.2	1.775	*p < 0.05*
PLHT	Plant height (cm)	132.70	23.81	8.2	6.895	***p < 0.001***
EHT	Ear height (cm)	68.20	19.74	9.6	5.224	**p < 0.001**
EPP	Ears per plant	1.30	0.89	10.9	1.645	*p < 0.05*
PASP	Plant aspect (1–5)	2.60	1.23	12.5	2.919	**p < 0.01**
EASP	Ear aspect (1–5)	2.20	1.16	11.1	2.634	**p < 0.01**

*LSD (0.05) = Least significant difference at 5% probability level; CV (%) = Coefficient of variation; Significance: p < 0.05, p < 0.01, *p < 0.001.*

3.5.2 Drought stress performance

Under drought conditions, grain yields among the fifty selected ranged from 0 to 2305.88 kg ha^{-1}, with a mean yield of 742.98 kg ha^{-1} (Table 3.7). Despite the moderate intensity of stress imposed, substantial genotypic variability was observed for all measured traits (Table 3.8). High-performing genotypes such as TZIL 25 (35G) 0.4 EMS, LMI 135 and EXP 124, exhibited superior yield, shorter anthesis-silking intervals (ASI ≤ 2 days), and higher leaf chlorophyll content (SPAD ≥ 35), indicating effective maintenance of photosynthetic efficiency under mositure stress.

The analysis of variance under drought indicated that there were significant ($p < 0.05$, $p < 0.01$ and $p < 0.001$) differences among the inbred lines for all traits except for ears per plant (EPP) (Table 3.8). For drought, H^2 estimates ranged from 0 for EPP to 46% for YLD (Table 3.8).

Table 3. 7: Performance of the best 20 and worst 10 inbred lines evaluated under drought environment in Legon, 2022.

Inbred	Grain yield (kg/ha)	Anthesis -silking interval	Leaf Chlorophyll content	Root lodging	Stalk lodging	Plant height (cm)	Ear height (cm)	Ears per plant
TZIL 25 (35G) 0.4EMS	2305.88	1	41.1	4	1	136.80	72.00	1
LMI 135	2055.58	1	42.4	4	1	124.40	63.50	1
EXP 124	1747.87	2	31.6	2	1	137.40	82.00	1
LMI 140	1584.06	1	26.8	4	1	141.80	67.10	1
TZIL 25 (60E) 0.4EMS	1542.73	2	38.8	1	1	141.00	67.70	1
TZIL 25 (40C) 0.4EMS	1438.17	3	39.2	4	1	139.80	67.30	2
DHL 166 POP 2	1258.13	0	23.6	7	1	130.40	57.40	1
DHL 101 POP 5	1249.41	-1	46.8	3	1	103.30	43.70	1
HP020-4/3/1-5/1	1203.70	3	31.7	2	1	106.80	49.70	1
DHL 258 POP 2	1019.75	5	35.1	4	1	105.80	48.00	1
TZIL 25 (43A) 0.4EMS	965.02	2	43.1	7	1	124.20	67.20	1
HP020-4/1(A)/1/1	881.99	-3	32.8	1	1	104.70	46.50	1
DHL 145 POP 2	765.88	5	28.4	3	1	117.40	57.20	1
DHL 184 POP 2	749.76	3	29.5	2	2	108.10	52.40	2
DHL 106 POP 5	654.90	0	7.8	6	1	109.25	46.25	1
HP020-5/2(A)/1/7	554.90	4	16.4	3	1	118.80	62.30	2
DHL 31 POP 1	498.04	1	16.4	8	1	111.55	66.65	1
LMI 141	498.04	1	38.4	2	1	94.80	51.30	2
1368	443.39	2	32.0	7	2	107.40	48.80	1
1368-250GY (21)B	407.84	3	30.0	7	1	119.30	56.20	1
DHL 142 POP 1	37.25	4	18.4	4	2	81.60	38.90	1
9006	35.29	4	5.4	2	2	95.20	44.20	1
DHL 44 POP 4	35.29	4	31.7	5	1	116.60	64.80	1
DHL 122 POP 1	33.33	5	23.1	4	1	125.70	59.20	1
DHL 18 POP 1	27.45	6	13.6	4	1	76.80	42.50	1
DHL 46 POP 1	23.53	3	17.5	3	1	144.60	72.40	1
DHL 71 POP 1	19.61	7	15.7	5	1	97.20	51.00	1
DHL 130 POP 3	0.00	0	19.3	2	1	102.37	60.70	1
DHL 223 POP 2	0.00	4	21.5	1	1	73.20	30.70	0
TZMI 760	0.00	0	26.3	1	1	63.21	21.38	1
Mean	**742.98**	**2**	**27.5**	**4**	**1**	**111.98**	**55.30**	**1**
Minimum	**0**	**-3**	**5.4**	**1**	**1**	**63.21**	**21.38**	**0**
Maximum	**2305.88**	**7**	**46.8**	**8**	**2**	**144.60**	**72.40**	**2**
S.E	**125**	**0**	**2**	**0**	**0**	**4**	**2**	**0**

Table 3. 8: Mean squares of the selected 50 maize inbred lines screened under drought environment at the WACCI Research Field, Legon during the harmattan season in 2022.

	Sources of variation				
Traits	**Gen**	**Rep**	**Rep(Blk)**	**Residual**	**H^2**
YLD	702038***	518577	40249	242554	46
DYANTH	208.119***	102.01	7.39	77.29	46
DYSK	297.4*	16.81	181.49	163.23	29
ASI	14.6531**	5.76	0.13	7.27	34
SPAD	217.14***	367.49*	78.33	66.48	47
RL	12.8973***	11.4	28.9772**	3.83	49
PLHT	699.78***	349.38	106.2	199.39	55
EHT	310.617***	176.98	71.56	124.65	43
EPP	0.29	0.43	0.02	0.46	0
Df	49	1	1	48	

Gen, genotypes; Rep, replication; Blk, Block; DYANTH, days to 50% anthesis; DYSK, days to 50%silking; ASI, anthesis-silking interval; SPAD, leaf chlorophyll content; RL, root lodging; SL, stalk lodging; PLHT, plant height; EHT, ear height; YLD, grain yield (kg/ha); EPP, ears per plant; Df, degrees of freedom.

'***' ,'**', '*' , = significance at p ($\propto = 0.05$), and p ($\propto = 0.01$) and p ($\propto = 0.001$) respectively.

3.5.3 Stress tolerance indices

Among the 100 inbred lines screened for herbicide tolerance, approximately 44% of the inbred lines had stress susceptibility index (SSI) value less than 1 (<1), suggesting varying degrees of tolerance to nicosulfuron application (Table 3.9). The most tolerant included DHL 65 POP 3, DHL 65 POP 5, DHL 77 POP 2, DHL 130 POP 3 and TZIL 25 (35G) 0.4 EMS, which maintained relatively high yield stability (YSI ≥ 1) despite herbicide exposure. Conversely, HP020-5/4/6/2, DHL 35 POP 1 and HP020-6/2(9)/9/6 recorded SSI value, 1.41, and YSI value, 0, indicating complete yield loss following herbicide application. Inbred lines such as C316-7, DHL 99 POP 2, LMI 140 and DHL 258 POP 2 combined moderate SSI values with YSI values, showing stable performance under both herbicide-treated and optimum conditions.

The computed stress susceptibility index (SSI) and yield stability index (YSI) for selected maize inbred lines are summarized in Tables 3.10 and 3.11. While yield ranges and means under the various environments have been discussed in earlier sections (3.5.1 and 3.5.2), these tables serve an analytical purpose, linking actual yield performance under stress and non-stress conditions to the derived tolerance indices. Their inclusion provides a clear representation of how genotype-specific yield responses informed the SSI and YSI calculations, thereby facilitating comparison of tolerance and yield stability among inbred lines across herbicide and drought environments.

Under the drought environment, most genotypes showed moderate to high susceptibility, SSI > 1.0, with only a few displaying tolerance. DHL 101 POP 5, EXP 124 and TZIL 25 (35G) 0.4 EMS exhibited negative SSI values with YSI > 1, signifying yield stability. Overall, SSI and YSI values under both stresses revealed susbstantial genotypic variability in response to the stress imposed, confirming that tolerance to herbicide and drought is genotype-dependent.

Table 3. 9: Inbred performance indices for herbicide stress susceptibility.

Inbred	YSI	SSI	GEN	YSI	SSI	Inbred	YSI	SSI	Inbred	YSI	SSI
1368	0.23	1.08	DHL 142 POP 1	0.71	0.41	DHL 31 POP 1	0.41	0.83	HP020-4/1(B)/1/1	0.00	1.41
9006	0.00	1.41	DHL 142 POP 5	0.44	0.79	DHL 35 POP 1	0.00	1.41	HP020-4/3/1-5/1	0.10	1.27
1368-150GY (119)S	0.48	0.74	DHL 145 POP 1	0.02	1.38	DHL 44 POP 4	0.65	0.49	HP020-4/4/1/6	0.12	1.24
1368-150GY(89)	0.32	0.95	DHL 145 POP 2	1.00	0.01	DHL 45 POP 4	0.09	1.29	HP020-5/1(B)/1/7	0.18	1.15
1368-250GY (21)B	0.51	0.69	DHL 148 POP 3	0.89	0.15	DHL 46 POP 1	0.32	0.96	HP020-5/2(A)/1/7	0.18	1.16
C316-7	0.49	0.72	DHL 148 POP 5	0.45	0.77	DHL 47 POP 3	0.20	1.13	HP020-5/3/2/3	0.04	1.35
CLRCYQ 40	0.78	0.31	DHL 156 POP 3	0.34	0.93	DHL 51 POP 2	0.21	1.11	HP020-5/4/6/2	0.00	1.41
DHL 101 POP 5	0.79	0.29	DHL 159 POP 1	0.29	1.00	DHL 53 POP 4	0.20	1.13	HP020-6/1(9)/1-7/2	0.00	1.41
DHL 104 POP 2	0.37	0.89	DHL 160 POP 2	0.69	0.43	DHL 60 POP 3	0.40	0.84	HP020-6/2(9)/9/6	0.00	1.41
DHL 106 POP 5	0.36	0.90	DHL 166 POP 2	0.26	1.04	DHL 65 POP 3	1.03	-0.04	LMI 102	0.03	1.36
DHL 107 POP 5	0.14	1.21	DHL 169 POP 2	0.29	1.00	DHL 65 POP 5	1.25	-0.36	LMI 135	0.28	1.01
DHL 109 POP 2	0.06	1.33	DHL 174 POP 2	0.00	1.41	DHL 71 POP 1	0.55	0.64	LMI 137	0.18	1.16
DHL 109 POP 3	0.30	0.98	DHL 179 POP3	0.00	1.41	DHL 71 POP 4	0.64	0.51	LMI 140	0.59	0.57
DHL 112 POP 3	0.00	1.41	DHL 18 POP 1	0.24	1.08	DHL 72 POP 5	0.31	0.98	LMI 141	0.22	1.10
DHL 116 POP 2	0.23	1.08	DHL 184 POP 2	0.74	0.37	DHL 77 POP 2	2.32	-1.86	LMI 144	0.23	1.08
DHL 119 POP 1	0.05	1.33	DHL 186 POP 2	0.34	0.94	DHL 81 POP 3	0.29	1.00	M131	0.23	1.08
DHL 119 POP 3	0.02	1.39	DHL 19 POP 3	0.23	1.09	DHL 86 POP 1	0.00	1.41	TZIL 25	0.00	1.41
DHL 121 POP 2	0.00	1.41	DHL 19 POP 5	0.38	0.87	DHL 89 POP 2	0.00	1.41	TZIL 25 (35G) 0.4EMS	4.29	-4.64
DHL 122 POP 1	0.11	1.25	DHL 194 POP 1	0.00	1.41	DHL 9 POP 3	0.72	0.39	TZIL 25 (40C) 0.4 EMS	0.06	1.32
DHL 130 POP 3	2.77	-2.50	DHL 21 POP 1	0.41	0.83	DHL 99 POP 2	0.83	0.24	TZIL 25 (43A) 0.4 EMS	0.45	0.78
DHL 131 POP 5	0.15	1.20	DHL 22 POP 3	0.05	1.34	EXP 124	0.31	0.97	TZIL 25 (60E) 0.4 EMS	0.62	0.53
DHL 132 POP 2	0.55	0.63	DHL 223 POP 2	0.31	0.97	HP020-1/3(10)/1-12/9	0.22	1.10	TZIL 25 (64D) 0.4 EMS	0.45	0.77
DHL 138 POP 5	0.28	1.02	DHL 224 POP 2	0.29	1.00	HP020-1/4/3/3	0.64	0.51	TZMI 760	0.30	0.99
DHL 139 POP 1	0.23	1.08	DHL 258 POP 2	0.44	0.79	HP020-2/5(9)/1/6	0.00	1.41	HP020-4/1(A)1/1	0.17	1.16
DHL 14 POP 1	0.00	1.41	DHL 29 POP 1	0.29	1.00						

YSI, yield stability index; SSI, stress susceptibility index.

Table 3. 10: Stress susceptibility index (SSI) and yield stability index (YSI) of best 20 and worst 10 inbred lines under herbicide and optimum conditions at Legon, 2022.

Inbred	HERB-grain yield (kg/ha)	OPT-grain yield (kg/ha)	Crop tolerance (7DAS)	Crop tolerance (21 DAS)	YSI	SSI
C316-7	1282.7	2610.63	4	3	0.49	0.72
DHL 99 POP 2	1226.5	1479.05	3	6	0.83	0.24
LMI 140	1210.1	2041.23	3	4	0.59	0.57
DHL 258 POP 2	1204.95	2735.25	2	2	0.44	0.79
DHL 145 POP 2	1168.45	1173.00	4	4	1	0.01
DHL 184 POP 2	1094.15	1478.93	2	4	0.74	0.37
TZIL 25 (43A) 0.4 EMS	1080.5	2424.75	4	3	0.45	0.78
TZIL 25 (60E) 0.4 EMS	1073.9	1719.62	5	4	0.62	0.53
DHL 142 POP 1	1056.25	1486.66	2	2	0.71	0.41
DHL 101 POP 5	969.8	1225.10	6	6	0.79	0.29
1368-250GY (21)B	949.2	1872.00	1	2	0.51	0.69
DHL 65 POP 5	947.8	755.23	2	2	1.25	-0.36
LMI 135	942.35	3308.25	3	4	0.28	1.01
DHL 186 POP 2	771.25	2297.39	4	2	0.34	0.94
1368-150GY(89)	763.7	2368.92	3	4	0.32	0.95
DHL 148 POP 5	735.05	1627.64	2	3	0.45	0.77
HP020-5/2(A)/1/7	716.45	3989.53	3	3	0.18	1.16
DHL 65 POP 3	672.35	652.74	4	3	1.03	-0.04
TZMI 760	625.95	2115.41	3	4	0.3	0.99
LMI 144	540.11	2306.32	3	3	0.23	1.08
1368	419.9	1813.16	3	4	0.23	1.08
DHL 194 POP 1	0	1921.21	7	9	0	1.41
DHL 35 POP 1	0	1046.18	4	7	0	1.41
DHL 86 POP 1	0	533.52	9	9	0	1.41
DHL 89 POP 2	0	1239.64	2	8	0	1.41
HP020-2/5(9)/1/6	0	1629.90	4	9	0	1.41
HP020-4/1(B)/1/1	0	3243.09	2	4	0	1.41
HP020-5/4/6/2	0	1090.19	7	9	0	1.41
HP020-6/1(9)/1-7/2	0	745.48	5	9	0	1.41
HP020-6/2(9)/9/6	0	2920.66	6	6	0	1.41
TZIL 25	0	2114.07	5	9	0	1.41
Mean	**639.84**	**1921.85**	**4**	**5**		
Minimum	**0**	**240.14**	**1**	**2**		
Maximum	**1282.7**	**3989.53**	**9**	**9**		
S.E	**91**	**228**	**0**	**0**		

HERB, herbicide-treated environment; OPT, optimal environment; YSI, yield stability index; SSI; stress susceptibility index; S.E, standard error.

Table 3. 11: Inbred performance indices for drought stress susceptibility.

Inbred	YSI	SSI	Inbred	YSI	SSI
1368	0.24	1.03	DHL 46 POP 1	0.01	1.35
9006	0.01	1.35	DHL 53 POP 4	0.04	1.32
1368-150GY (119)S	0.22	1.07	DHL 65 POP 5	0.40	0.82
1368-150GY(89)	0.07	1.27	DHL 71 POP 1	0.02	1.34
1368-250GY (21)B	0.22	1.07	DHL 99 POP 2	0.10	1.23
C316-7	0.13	1.20	EXP 124	1.25	-0.34
DHL 101 POP 5	1.02	-0.03	HP020-1/3(10)/1-12/9	0.04	1.32
DHL 106 POP 5	0.45	0.76	HP020-4/1(A)1/1	0.35	0.89
DHL 107 POP 5	0.25	1.02	HP020-4/3/1-5/1	0.36	0.88
DHL 122 POP 1	0.01	1.35	HP020-4/4/1/6	0.12	1.20
DHL 130 POP 3	0.00	1.37	HP020-5/1(B)/1/7	0.05	1.30
DHL 139 POP 1	0.03	1.33	HP020-5/2(A)/1/7	0.14	1.18
DHL 142 POP 1	0.03	1.34	HP020-5/3/2/3	0.13	1.19
DHL 145 POP 1	0.46	0.74	HP020-6/2(9)/9/6	0.13	1.20
DHL 166 POP 2	0.85	0.21	LMI 102	0.01	1.35
DHL 18 POP 1	0.01	1.35	LMI 135	0.62	0.52
DHL 184 POP 2	0.51	0.68	LMI 140	0.78	0.31
DHL 186 POP 2	0.15	1.17	LMI 141	0.22	1.08
DHL 194 POP 1	0.04	1.31	LMI 144	0.05	1.31
DHL 223 POP 2	0.00	1.37	M131	0.10	1.24
DHL 224 POP 2	0.21	1.08	TZIL 25 (35G) 0.4EMS	9.53	-11.69
DHL 258 POP 2	0.47	0.73	TZIL 25 (40C) 0.4 EMS	0.41	0.80
DHL 31 POP 1	0.31	0.94	TZIL 25 (43A) 0.4 EMS	0.40	0.82
DHL 44 POP 4	0.06	1.29	TZIL 25 (60E) 0.4 EMS	0.90	0.14
DHL 45 POP 4	0.06	1.28	TZMI 760	0.00	1.37

YSI, yield stability index; SSI; stress susceptibility index (SSI).

Table 3. 12: Stress susceptibility index (SSI) and yield stability index (YSI) of selected maize inbred lines under drought and optimum conditions at Legon, 2022.

Inbred	DRT-Grain yield (kg/ha)	OPT- Grain yield (kg/ha)	YSI	SSI
LMI 135	2055.58	3308.25	0.62	0.52
EXP 124	1747.87	1400.84	1.25	-0.34
LMI 140	1584.06	2041.23	0.78	0.31
TZIL 25 (60E) 0.4EMS	1542.73	1719.62	0.9	0.14
TZIL 25 (40C) 0.4EMS	1438.17	3484.62	0.41	0.95
DHL 258 POP 2	1019.07	2735.25	0.47	0.73
DHL 166 POP 2	1258.13	1480.6	0.85	0.21
DHL 101 POP 5	1249.41	1225.1	1.02	-0.03
HP020-4/3/1-5/1	1203.7	3370.75	0.36	0.88
TZIL 25 (43A) 0.4EMS	965.02	2424.75	0.4	0.82
HP020-4/1(A)/1/1	881.99	2518.48	0.35	0.89
DHL 145 POP 2	765.88	1172.99	0.65	0.56
DHL 184 POP 2	749.76	1478.93	0.51	0.68
DHL 106 POP 5	654.9	1470.57	0.45	0.76
HP020-5/2(A)/1/7	554.9	3989.53	0.14	1.18
DHL 31 POP 1	498.04	1598.82	0.31	0.94
LMI 141	498.04	2315.96	0.22	1.08
1368	443.39	1813.16	0.24	1.03
1368-250GY (21)B	407.84	936	0.22	1.07
TZIL 25	145.09	1132.86	0.12	1.42
DHL 142 POP 1	37.25	1486.66	0.03	1.59
9006	35.29	2825.07	0.01	1.35
DHL 44 POP 4	35.29	263.18	0.13	1.41
DHL 122 POP 1	33.33	3064.85	0.01	1.61
DHL 18 POP 1	27.45	2058.98	0.01	1.61
DHL 46 POP 1	23.53	1604.87	0.02	1.61
DHL 71 POP 1	19.61	997.9	0.02	1.59
DHL 130 POP 3	0	778.05	0	1.63
DHL 223 POP 2	0	622.15	0	1.63
TZMI 760	0	2115.41	0	1.63
Mean	**742.98**	**1921.85**		
Minimum	**0**	**240.14**		
Maximum	**2305.88**	**3989.53**		
Standard error	**125**	**228**		

3.5.4 Genetic correlations among traits under environments

Under the herbicide-treated environment , grain yield (YLD) was moderately positively correlated with plant height (PLHT, $r = 0.46$) and ear height (EHT, $r = 0.42$) (Figure 3.2). These associations indicate that taller plants tended to maintain higher yields even under nicosulfuron stress. In contrast, grain yield exhibited strong negative correlations with ear aspect (EASP, $r = -0.58$) and plant aspect (PASP, $r = -0.41$), implying that poor ear and plant architecture were associated with reduced yield. Negative correlations were also observed between grain yield and crop tolerance scores, both at 7 days after spraying (CT1, $r = -0.22$) and 21 days after spraying (CT2, $r = -0.37$), reflecting that genotypes showing greater visible injury tended to produce lower grain yield.

There was a strong positive correlation between plant height and ear height ($r = 0.6$), indicating consistent plant stature relationships, and between days to anthesis (DYANTH) and days to silking (DYSK) ($r = 0.62$), confirming synchrony between male and female flowering under herbicide stress conditions.

Across environments (Figure 3.3), there was a strong positive correlation between plant height and ear height ($r = 0.74$) and days to anthesis and days to silking ($r = 0.71$). A moderate positive correlation was observed between plant aspect and ear aspect ($r = 0.34$), suggesting that phenotypic appearance traits were generally consistent across environments. Overall, low to moderate genetic associations were observed between grain yield and other measured traits, highlighting the complex and multigenic nature of yield expression under varying environmental conditions.

Under drought stress (Figure 3.4), there was a strong positive correlation between plant height and ear height ($r = 0.85$), reflecting consistent growth patters under water deficit. In addition, grain yield was positively correlated with plant height ($r = 0.56$) and ear height ($r = 0.45$), indicating

that taller plants with higher ear placement performed better under drought. A positive correlation was also observed between grain yield and leaf chlorophyll content (SPAD, $r = 0.51$), indicating that genotypes with higher leaf cholorophyll content had better photosynthetic capacity and yield under moisture stress.

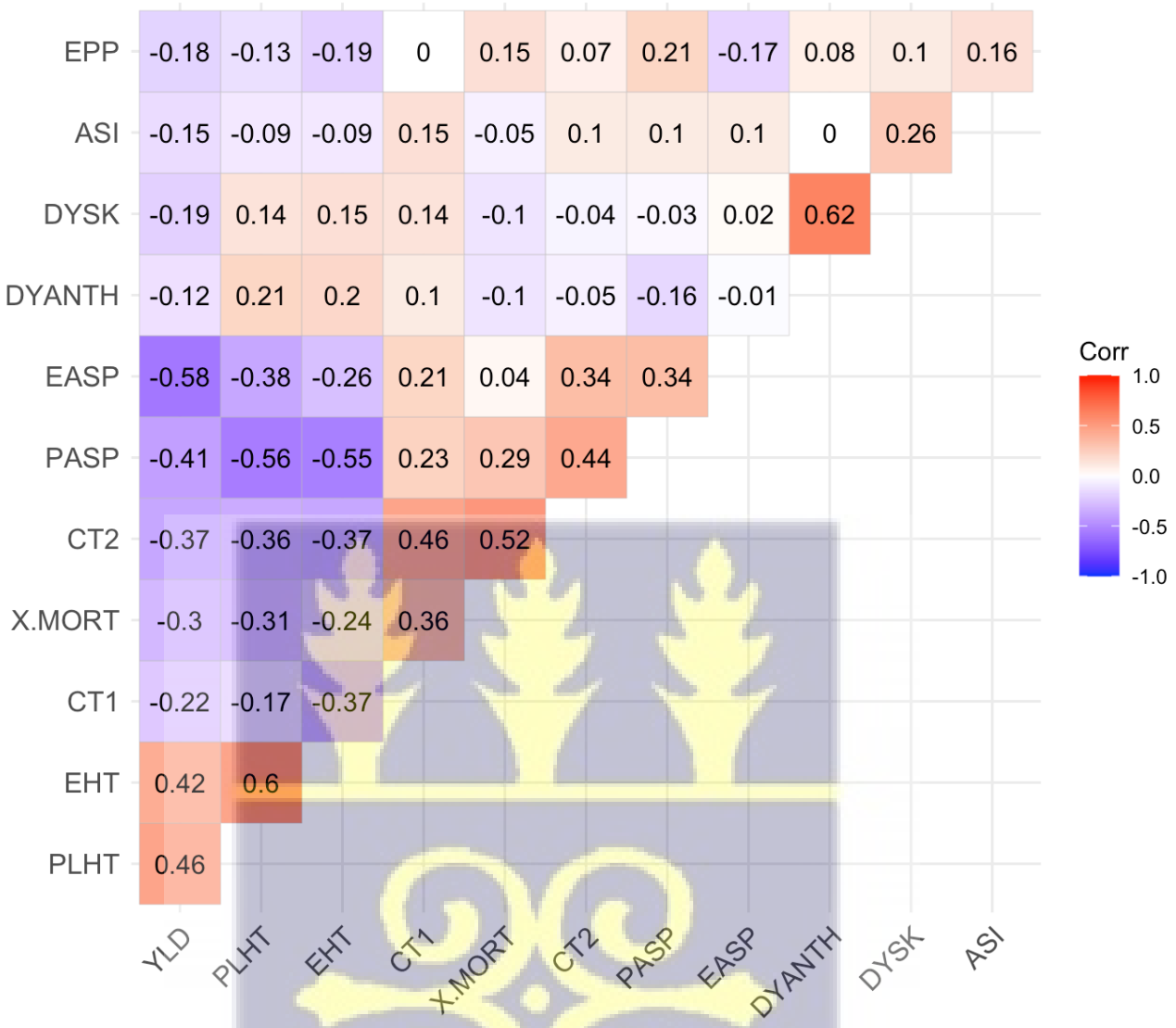

Figure 3. 2: Genetic correlations among traits under herbicide-treated environment in WACCI Research Field, 2022.

(*CT1, crop tolerance at 7 days after spraying (DAS); CT2, crop tolerance at 21 DAS; DYANTH, days to 50% anthesis; DYSK, days to 50%silking; ASI, anthesis-silking interval; PLHT, plant height; EHT, ear height; EAS, ear aspect; YLD, grain yield (kg/ha)*

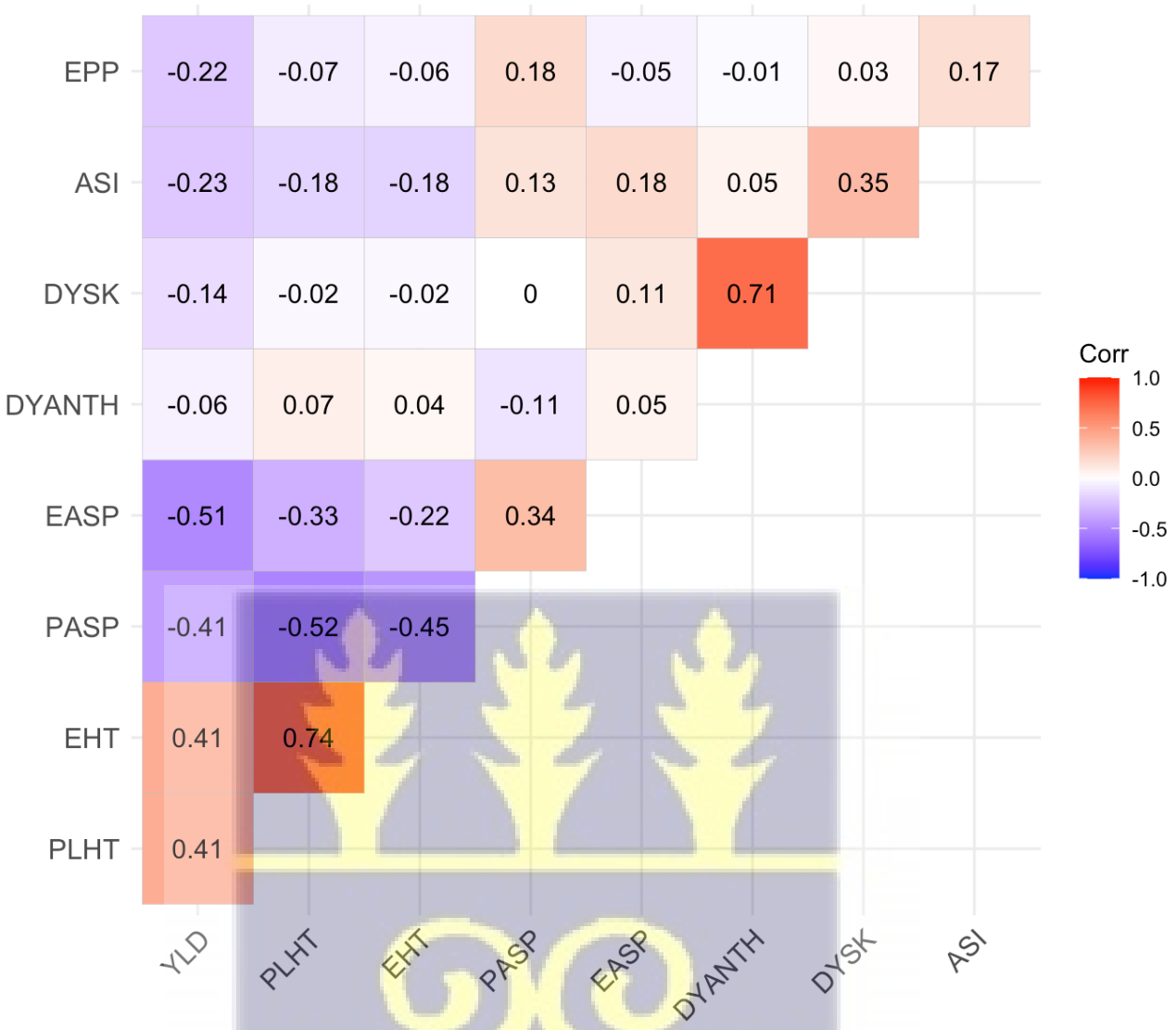

Figure 3. 3: Genetic correlations among grain yield and related traits across environments in WACCI Research Field, 2022.

(DYANTH, days to 50% anthesis; DYSK, days to 50%silking; ASI, anthesis-silking interval; PLHT, plant height; EHT, ear height; EASP, ear aspect; YLD, grain yield (kg/ha)

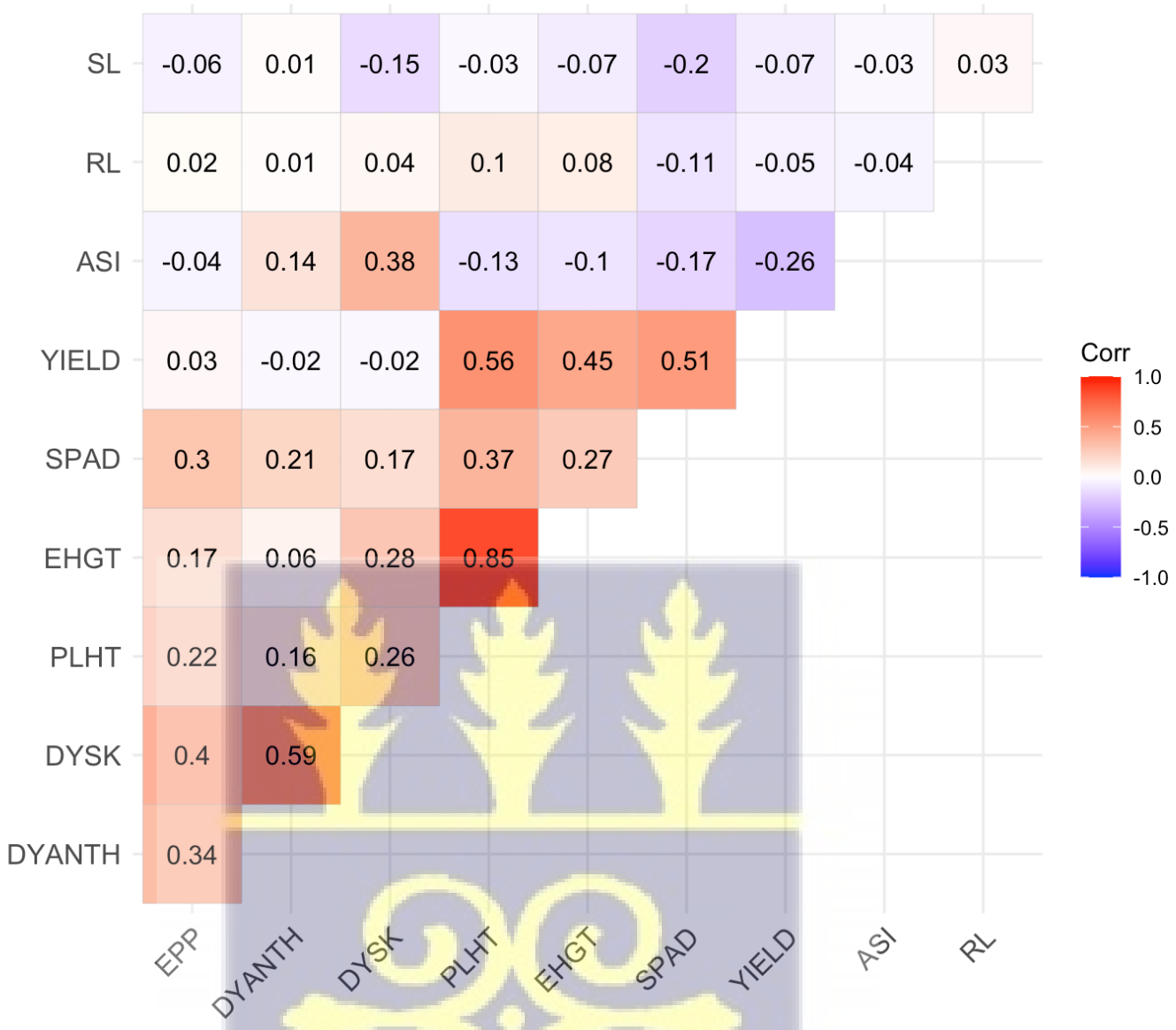

Figure 3. 4: Genetic correlations among grain yield and other traits under drought in WACCI Research Field, 2022.

(DYANTH, days to 50% anthesis; DYSK, days to 50%silking; ASI, anthesis-silking interval; SPAD, leaf chlorophyll content; RL, root lodging; PLHT, plant height; EHT, ear height; YLD, grain yield (kg/ha); EPP, ears per plant.)

3.5.3 BLUPs-based performance of inbreds

The best linear unbiased predictions (BLUPs) for grain yield were used to estimate the breeding values of the maize inbred lines across environments (Table 3.10). Under the herbicide-treated environment, the top 10 outstanding inbreds for grain yield included C316-7 (889 kg ha^{-1}), DHL 258 POP 2 (863 kg ha^{-1}), LMI 140 (835 kg ha^{-1}), DHL 145 POP 2 (827 kg ha^{-1}), DHL 184 POP 2 (779 kg ha^{-1}), TZIL 25 (43A) 0.4 EMS (760 kg ha^{-1}), TZIL 25 (60E) 0.4 EMS (751 kg ha^{-1}), TZIL 25 (35G) 0.4 EMS (717 kg ha^{-1}), LMI 135 (694 kg ha^{-1}) and 1368-250GY (21)B (687 kg ha^{-1}). These genotypes maintained relatively higher yields under herbicide application, suggesting partial tolerance to nicosulfuron stress.

Under the optimum environment, the top 10 performing inbreds for grain yield were TZIL 25 (40C) 0.4 EMS (2961 kg ha^{-1}), HP020-4/3/1-5/1 (2809 kg ha^{-1}), DHL 122 POP 1 (2583 kg ha^{-1}), 9006 (2438 kg ha^{-1}), DHL 258 POP 2 (2835 kg ha^{-1}), LMI 102 (2379 kg ha^{-1}), C316-7 (2235 kg ha^{-1}), HP020-4/1(A)/1/7 (2224 kg ha^{-1}), HP020-5/2(A)/1/7 (2117 kg ha^{-1}) and TZIL 25 (43A) 0.4 EMS (2107 kg ha^{-1}). These results highlight that several genotypes performed well under both stress and non-stress conditions, with DHL 258 POP 2, C316-7 and TZIL 25 (43A) 0.4 EMS consistently being among the top inbreds.

Across the two environments, the top 10 outstanding inbreds included DHL 258 POP 2 (1287 kg ha^{-1}), C316-7 (1277 kg ha^{-1}), TZIL 25 (43A) 0.4 EMS (1200 kg ha^{-1}), LMI 140 (1150 kg ha^{-1}), HP020-5/2(A)/1/7 (1131 kg ha^{-1}), DHL 186 POP 2 (1113 kg ha^{-1}), LMI 135 (1107 kg ha^{-1}), TZIL 25 (40 C) 0.4 EMS (1097 kg ha^{-1}), HP020-4/3/1-5/1 (1093 kg ha^{-1}) and LMI 102 (1071 kg ha^{-1}). The consistent presence of several of these genotypes across environments suggests broad adaptability and stable yield potential.

Under drought stress, the top 10 inbreds for grain yield based on BLUP-estimated grain yield were TZIL 25 (35G) 0.4 EMS (1776 kg ha^{-1}), TZIL 25 (60E) 0.4 EMS (1149 kg ha^{-1}), DHL 258 POP 2 (950 kg ha^{-1}), EXP 124 (831 kg ha^{-1}), DHL 101 POP 5 (829 kg ha^{-1}), DHL 166 POP 2 (800 kg ha^{-1}), DHL 184 POP 2 (709 kg ha^{-1}), LMI 140 (659 kg ha^{-1}), DHL 106 POP 5 (624 kg ha^{-1}) and TZIL 25 (40C) 0.4 EMS (593 kg ha^{-1}). These genotypes maintained high BLUPs even under water deficit, demonstrating genetic potential for drought resilience.

Overall, results revealed significant differences in BLUP-based yield estimates among the maize inbred lines subjected to herbicide-treated and manual weeding with observed low breeding values of the inbreds under herbicide-control in comparison to those estimated under manual weeding (Table 3.10). This indicates the strong depressive effect of herbicide stress on yield expression and the presence of genotype-specific tolerance mechanisms among certain inbred lines such as DHL 258 POP 2, C316-7 and TZIL 25 (43A) 0.4 EMS.

Table 3. 13: BLUPs-based yield performance of the top 20 maize inbred lines under herbicide-control, optimum, across environments, and under drought stress at WACCI Research Field, Legon, 2022.

Inbred	Herbicide ($kgha^{-1}$)	Inbred	Manual weeding ($kgha^{-1}$)	Inbred	Across ($kgha^{-1}$)	Inbred	Drought ($kgha^{-1}$)
C316-7	889	TZIL 25 (40C) 0.4 EMS	2961	DHL 258 POP 2	1287	TZIL 25 (35G) 0.4EMS	1776
DHL 258 POP 2	863	HP020-4/3/1-5/1	2809	C316-7	1277	TZIL 25 (60E) 0.4EMS	1149
LMI 140	835	DHL 122 POP 1	2583	TZIL 25 (43A) 0.4 EMS	1200	DHL 258 POP 2	950
DHL 145 POP 2	827	9006	2438	LMI 140	1150	EXP 124	831
DHL 184 POP 2	779	DHL 258 POP 2	2385	HP020-5/2(A)/1/7	1131	DHL 101 POP 5	829
TZIL 25 (43A) 0.4 EMS	760	LMI 102	2379	DHL 186 POP 2	1113	DHL 166 POP 2	800
TZIL 25 (60E) 0.4 EMS	751	C316-7	2235	LMI 135	1107	DHL 184 POP 2	709
TZIL 25 (35G) 0.4EMS	717	HP020-4/1(A)1/1	2224	TZIL 25 (40C) 0.4 EMS	1097	LMI 140	659
LMI 135	694	HP020-5/2(A)/1/7	2117	HP020-4/3/1-5/1	1093	DHL 106 POP 5	624
1368-250GY (21)B	687	TZIL 25 (43A) 0.4 EMS	2107	LMI 102	1071	TZIL 25 (40C) 0.4EMS	593
DHL 65 POP 5	663	DHL 53 POP 4	2052	LMI 144	1069	HP020-5/2(A)/1/7	534
1368-150GY (119)S	631	LMI 144	2040	9006	1065	DHL 31 POP 1	483
1368-150GY(89)	608	DHL 186 POP 2	2033	LMI 141	1064	LMI 141	483
DHL 186 POP 2	592	LMI 141	2031	TZIL 25 (60E) 0.4 EMS	1058	TZIL 25 (43A) 0.4EMS	437
DHL 148 POP 5	576	TZMI 760	1907	TZMI 760	1048	1368	434
DHL 223 POP 2 (A)	558	DHL 119 POP 3	1866	1368-250GY (21)B	1047	1368-250GY (21)B	403
HP020-5/2(A)/1/7	549	LMI 140	1853	DHL 184 POP 2	1015	DHL 107 POP 5	385
DHL 65 POP 3	542	LMI 135	1851	DHL 18 POP 1	1009	HP020-4/1(A)/1/1	376
DHL 31 POP 1	533	DHL 194 POP 1	1839	1368-150GY (119)S	998	1368-150GY (119)S	369
TZMI 760	533	DHL 18 POP 1	1837	DHL 148 POP 5	973	HP020-6/2(9)/9/6	368

3.5.4 Genetic diversity and ISSR polymorphism

Out of the 30 ISSR primers tested, only 10 primers displayed clear and reproducible amplification bands across the fifty (50) maize inbred lines (Table 3.14). These primers generated a total of 52 polymorphic loci, ranging from 2 loci (ISSR-05) to 7 loci (ISSR-06, and ISSR-11), with an average of 5 loci per primer. All 10 selected primers showed 100% polymorphism across the 50 genotypes, indicating their high discriminative ability in detecting genetic variation among the inbred lines.

The genetic diversity values among markers ranged from 0.24 for ISSR-05 to 0.81 for ISSR-06 with an average of 0.62. Locus presence frequency ranged from 0.26 for ISSR-11 to 0.66 for ISSR-05 with an average of 0.51, representing the proportion of amplified loci detected across all genotypes.

Among the markers, ISSR-06 was the most informative and polymorphic marker with the highest PIC (0.78), maximum number of alleles (7), and the highest heterozygosity (0.71). It also reported the highest gene diversity of 0.81. In contrast, ISSR-05 displayed the lowest PIC (0.21), the fewest alleles (2) and the lowest heterozygosity (0.03) despite having the highest minor allele frequency (0.86). Notably, 70% of the ISSR markers showed PIC value ≥ 0.50, while 40% exhibited heterozygosity value ≥ 0.50. These results highlight the informativeness and reliability of the selected markers for diversity assessment.

The genetic similarity coefficient among the 50 inbred lines, estimated using Jaccard's similarity index, ranged from 0.1 to 1 (Figure 3.5). The highest similarity coefficient was found between genotypes 17 (DHL 166 POP 2) and 3 (DHL 106 POP 5), whereas the lowest similarity

was found between genotypes 48 (TZMI 760) and 15 (LMI 140), showing genetic variation among the 50 maize genotypes used in the study.

Cluster analysis using the unweighted pair group method with arithmetic mean (UPGMA) grouped the 50 inbred lines into four distinct clusters (Figure 3.6). The largest cluster consisted 47 inbred lines , while three inbred lines, TZIL 25 (white kernel), DHL 18 POP 1 (yellow kernel) and 1368-150 GY (119)S (white kernel), formed single-member clusters. No distinct cluttering pattern was detected based on endosperm colour or source populations, indicating that genetic relatedness among lines was independent of kernel colour or origin.

Table 3. 14: Genetic diversity parameters and polymorphism information content of the 10 ISSR markers.

Marker	Minor allele frequency	Polymorphic loci	Locus presence frequency	Gene Diversity	Heterozygosity	PIC	% Polymor-phism
ISSR-05	0.86	2.0	0.66	0.24	0.03	0.21	100
ISSR-06	0.31	7.0	0.62	0.81	0.71	0.78	100
ISSR-08	0.44	5.0	0.54	0.70	0.26	0.65	100
ISSR-09	0.28	6.0	0.54	0.79	0.56	0.75	100
ISSR-10	0.29	5.0	0.48	0.78	0.58	0.74	100
ISSR-11	0.39	7.0	0.26	0.73	0.69	0.69	100
ISSR-25	0.31	6.0	0.48	0.74	0.38	0.69	100
ISSR-26	0.76	5.0	0.62	0.40	0.13	0.38	100
ISSR-39	0.80	4.0	0.40	0.34	0.20	0.32	100
ISSR-43	0.44	4.0	0.50	0.69	0.24	0.63	100
Mean	**0.49**	**5.1**	**0.51**	**0.62**	**0.38**	**0.58**	

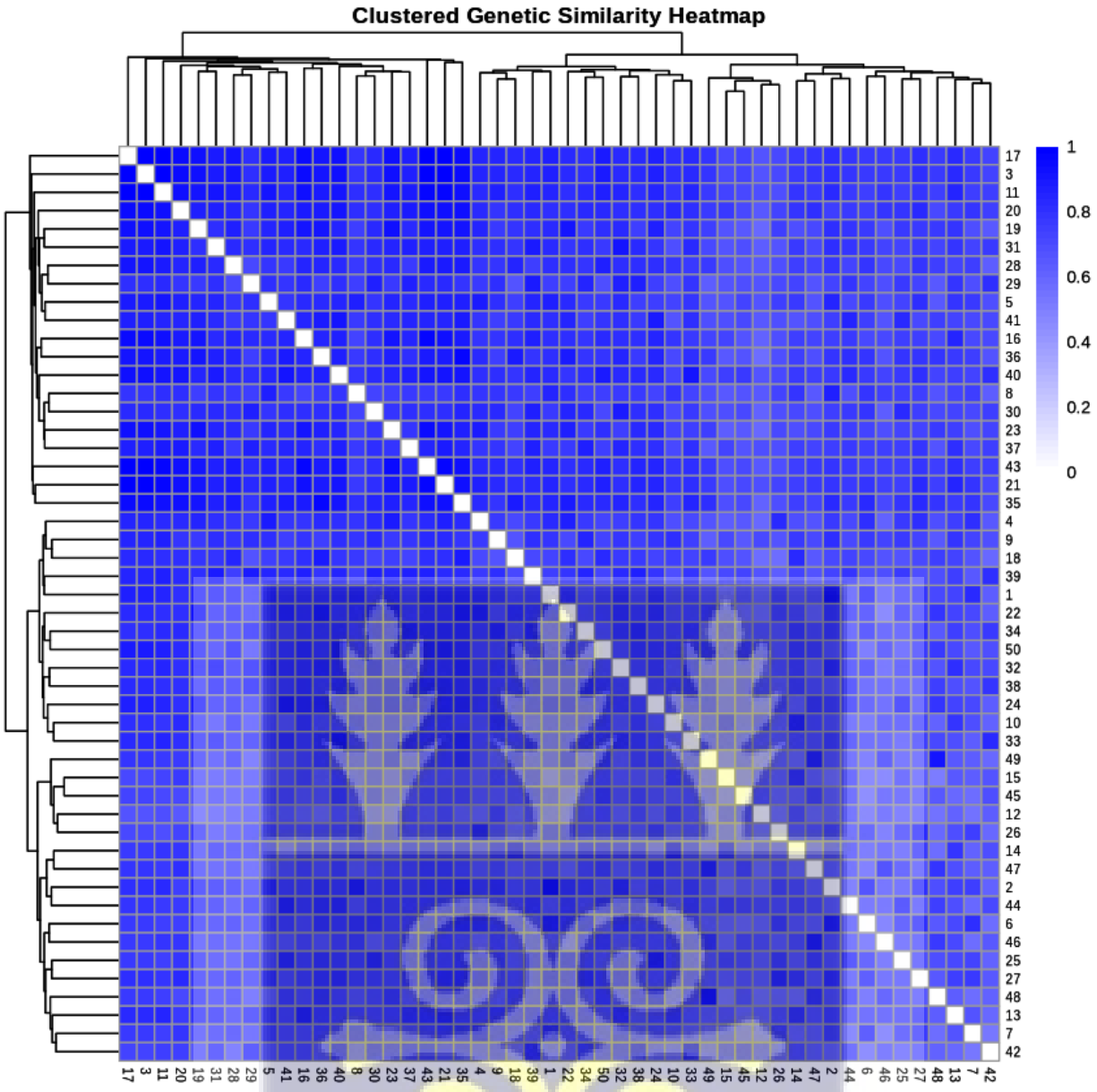

Figure 3. 5: Genetic similarity heatmap showing pairwise genetic relationships among 50 maize inbred lines based on 10 polymorphic ISSR markers.

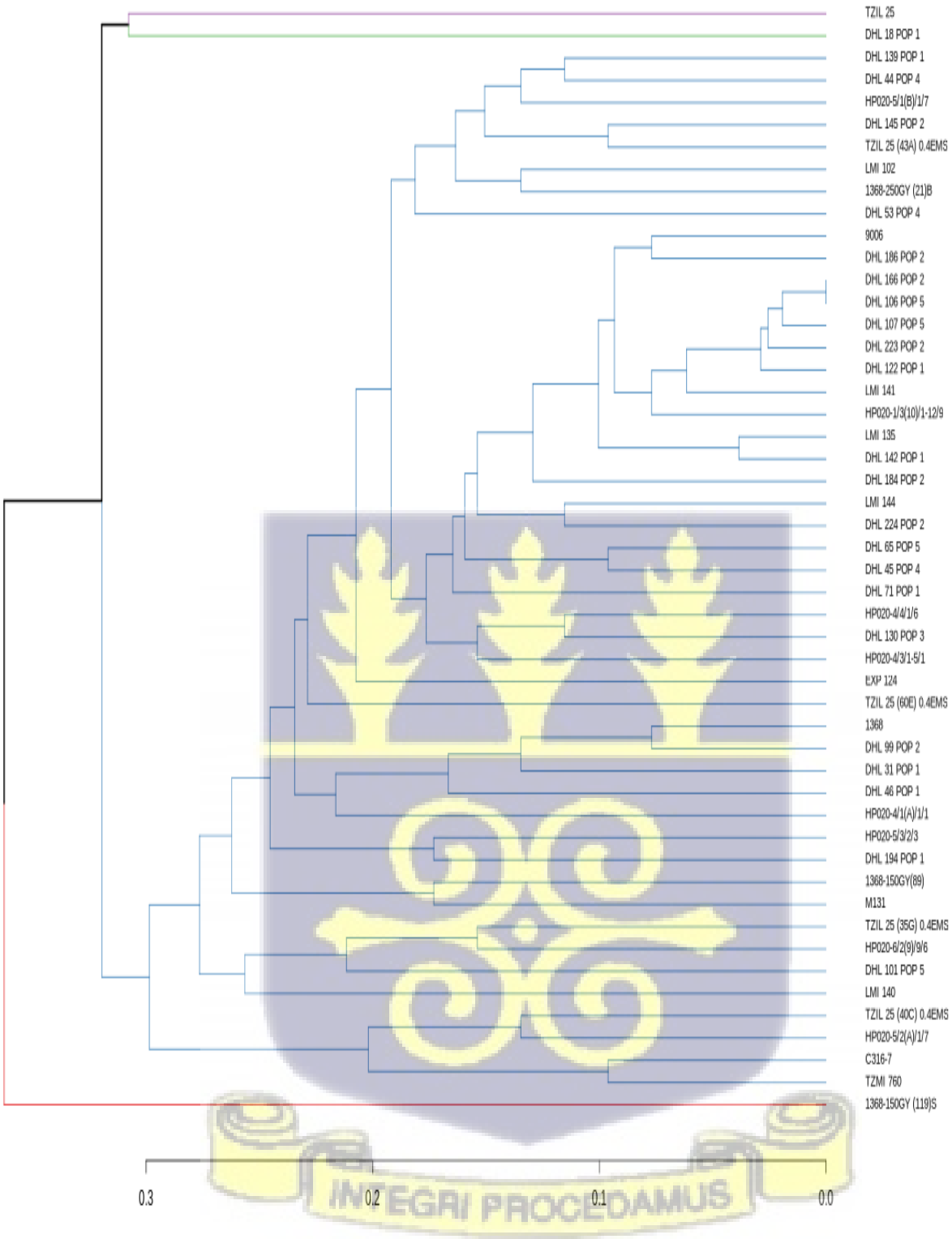

Figure 3. 6: UPGMA-dendrogram based on Jaccard's similarity coefficient depicting the genetic relationships among 50 maize inbred lines.

3.6 Discussion

Genetic variability is essential for progress from selection for improvement of agricultural traits under varying environments. The disparity observed in grain yield under the herbicide-treated environment, from 0 for several inbreds to a maximum of 1282.7 kg for C316-7, underscores the importance of selecting inbred lines that can maintain productivity despite herbicide-induced stress. The presence of both high-yielding and low-yielding inbreds indicates a potential for genetic improvement through selection (Nyasha *et al.*, 2018). Despite herbicide damage, measured through crop tolerance assessed at two intervals using a tolerance rating scale from 1 – 9, inbred lines such as C316-7 and DHL 99 POP 2, exhibited high grain yield. This suggests that inherently, these inbred lines may have genes that confer herbicide tolerance to the herbicide, allowing them to maintain productivity despite the stress. Previous studies show that maize genotypes differ in their inherent ability to withstand herbicides, for example, Nyasha *et al.* (2018) in applying certain herbicide combinations on maize inbred lines observed severe phytotoxicity and yield losses thereby classifying them as susceptible inbreds, whereas other inbreds were classified as herbicide-tolerant with minimal yield penalties. These findings reinforce that screening and selecting maize inbred lines for herbicide tolerance is crucial for breeding programmes aiming to maintain yields in weed-management systems that rely on chemical control.

Herbicide damage in the study was quantified using crop injury ratings and plant mortality counts at two intervals after spraying. Spraying was done at two different times which coincided with various crop growth stages, with the first spraying taking place two weeks after planting (V1growth stage) and the second spraying done at the tasseling stage (V10 – VT growth stage). Across most inbred lines, the mortality rates varied significantly and increased over time with 21

days after first spraying showing the highest mortality percentages. Some inbred lines such as DHL 140 POP 1, HP020-5/2(A)/1/7, DHL 65 POP 5, and C316-7 displayed 3-7% mortality by 21 days after the first spraying while other such as DHL 130 POP 3 and 1368-150GY (89) reached 46-73% mortality by the same time interval. The variation in mortality rates across maize inbred lines following herbicide application can be attributed to both inherent genetic factors and the time-dependent physiological responses of plants to herbicides. Research indicates that inbred lines exhibit significant variability in their sensitivity to herbicides, often influenced by specific genetic factors associated with herbicide resistance. Y. Zhang *et al.* (2024) reported that specific maize inbred lines such as J1399 and 80162 exhibited significant susceptibility to nicosulfuron while tolerance is often associated with the presence of certain resistant genes. Other studies also discuss how various genetic backgrounds can lead to differential expression of herbicide tolerance traits, aligning with the time-dependent nature of herbicide effects on plant physiology (Bonis *et al.*, 2011; Stefanović *et al.*, 2010). Research have also shown that certain inbred lines possess unique genetic traits, such as DNA methylation changes that influence the expression of resistance genes, enhancing their ability to detoxify herbicides thereby lowering mortality rates (Mahmoud *et al.*, 2020; Tyczewska *et al.*, 2021). Maize inbred lines that survived likely possess inherent genetic factors to metabolize or compartmentalize nicosulfuron thereby preventing lethal damage.

In the application of post-emergence herbicides to control weeds, it is important to note the crop growth stage at which there will be higher phytotoxicity to determine the best time to apply the herbicide. In this study, DHL 130 POP 3 displayed a mortality of 41% at 7 days after spraying, escalating to 73% by 21 days post-application. This pattern suggests a delayed response to herbicide stress, where plants initally tolerate herbicide exposure before exhibiting significant

mortality at a later stage (Galon *et al.*, 2020). Such delayed responses can be attributed to the time required for the herbicide to disrupt physiological processes within the plant, leading to visible symptoms and ultimately higher mortality rates as time progresses. In contrast, DHL 99 POP 2 showed no mortality at 7 days after spraying but increased to 23% by 21 days after spraying which may indicate a threshold response to herbicide exposure, where the inbred line initially shows resistance to the herbicide but eventually shows susceptibility over a period. This phenomenon is essential for understanding the appropriate timing of herbicide application. The findings from this study tentatively suggest that the V10 – VT stage was less lethal to the inbred lines, however further research and agronomic practices would be required before altering herbicide timing recommendations. Additionally, it has been noted that judicious timing of herbicide applications across different growth stages is crucial for maintaining crop viability and optimizing weed control outcomes (Norsworthy *et al.*, 2012).

Beyond the herbicide treatment, the inbred lines were evaluated under an optimal environment where weeds were controlled manually and under drought-stress environment. This allowed the identification of genotypes with broad or specific adaptation. Notably, certain inbred lines consistently ranked among the top performers across multiple environments, underscoring their resilience. These include, C316-7, DHL 258 POP 2 and TZIL 25 (60E) 0.4 EMS were high performers under herbicide stress, under optimal (manual weeding) conditions, and , for the latter two, even under drought. Such stable performance across diverse stress and non-stress conditions indicates broad adpatability and suggests these inbreds carry a combination of desirable alleles for yield potential and stress tolerance (Menkir *et al.*, 2024b; Obeng-Bio *et al.*, 2020b; F. Zhang *et al.*, 2021). These widely adapted inbreds are valuable in breeding programmes focused on improving yield stability under varying climatic conditions and management practices.

Conversely, some inbreds were top performers only in specific environments. It is notable that C316-7, the highest yielder under herbicide, remained among the top performers in optimal conditions implying that its high yield potential is not solely due to herbicide tolerance but inherent genetic robustness. In contrast, other inbred lines had no yields with complete crop failure under herbicide but produced yield under manual weeding, confirming their suscpetibility to nicosulfuron. This reinforces the importance of herbicide screening of maize genotypes.

In assessing the tolerance of maize inbred lines under varying stress conditions, quantitative evaluation using stress indices such as the Yield Stability Index (YSI) and the Stress Susceptibility Index (SSI) was used. These metrics are essential for evaluating the performance stability of these genotypes in response to adverse environmental conditions (Osuman *et al.*, 2020). YSI is designed to measure the stability of yield across different environments, with higher values indicating greater stability and adaptability of the genotype under varied conditions. Conversely, the SSI quatifies the susceptibility of genotypes to stress, where lower SSI values indicate greater stress tolerance (Sharma, *et al.*, 2023). Also indices like YSI and SSI facilitate the comparison of inbred lines, allowing breeders to identify and select genotypes that not only achieve high yields under optimal conditions but also exhibit resilience when subjected to stresses (Feizi *et al.*, 2020). The high YSI value (>1) and low SSI value (<1) revealed DHL 65 POP 3, DHL 66 POP 5, DHL 77 POP 2, DHL 130 POP 3 and TZIL 25 (35G) 0.4 EMS as highly tolerant to nicosulfuron. Other inbred lines with SSI value <1 identified to be tolerant include C316-7, DHL 258 POP 2, TZIL 25 (60E) 0.4 EMS, DHL 99 POP 2 and HP020-1/4/3/3. Under drought environment, DHL 101 POP 5, EXP 124 and TZIL 25 (35G) 0.4 EMS with high YSI value (>1) and low SSI value (<1) were identified to be drought tolerant. Other drought-tolerant inbred lines with SSI value <1 include DHL 31 POP 1, HP020-4/1(A)/1/1, HP020-4/3/1-

5/1, TZIL 25 (43A) 0.4 EMS, DHL 65 POP 5, TZIL 25 (40C) 0.4 EMS, DHL 106 POP 5, DHL 145 POP 1, DHL 258 POP 2, DHL 184 POP 2, LMI 135, LMI 140, DHL 166 POP 2 and TZIL 25 (60E) 0.4 EMS.

The application of Best Linear Unbiased Prediction (BLUP) in plant breeding, enhances the ability to evaluate the performance by providing a comprehensive approach that incorporates both fixed and random effects. BLUP provides breeders a more comprehensive prediction of genotypic performance while accounting for inherent variability among individual plants, which can be significant in heterogenous environment (Woyann *et al.*, 2020). In comparing the top performers identified by environment-specific mean yields and BLUP-based estimates, there was considerable agreement. Notably, under the herbicide-stress environment, C316-7, DHL 258 POP 2, LMI 140, DHL 145 POP 2, DHL 184 POP 2, TZIL 25 (43A) 0.5 EMS AND TZIL 25 (60E) 0.4 EMS were selected both by the raw means and BLUPs as elite performers. TZIL 25 (40C) 0.4 EMS, 9006, LMI 102 and DHL 258 POP 2 were the four of the top ten performers selected by both methods under manual weeding environment. Under drought, TZIL 25 (35G) 0.4 EMS, EXP 124, LMI 140, TZIL 25 (60E) 0.4 EMS, TZIL 25 (40C), DHL 166 POP 2 and DHL 101 POP 5 were selected by both methods among the top ten performers. Notably, DHL 258 POP 2 was consistently selected among the top ten performers in all environments by both methods, reinforcing its status as a broadly superior line. The general concordance betweem BLUP-bsed and mean-based selection in the study suggests that genotype rankings were robust. However, the inclusion of BLUPs adds value by accounting for variance and providing shrinkage etsimates, which is beneficial in scenarios where the performance data are unbalanced or when environmental variances are pronounced (Anuradha *et al.*, 2022). By addressing these statistical

nuances, BLUPs facilitate a more accurate depiction of genotypic performance (Hossain *et al.*, 2023).

Analysis of variance revealed significant genotypic mean squares ($p < 0.001$) for grain yield and most measured traits under herbicide-treated, manual weeding, drought and across all environments. This is an indication of genetic variability among the inbred lines used for the study. In the herbicide-stress environment, the genotypic effect was significant for plant mortality due to the herbicide sprayed, indicating that the inbred lines differed in their response to nicosulfuron. This implies that some inbred lines carry alleles for herbicide tolerance while others are genetically susceptible, an important variation that must be considered by breeders. This result is corroborated by Sakadzo *et al.*, (2018) who reported significant genotype by herbicide interaction for maize traits highlighting that different herbicide treatments caused varying levels of phyotoxicity, changes in ASI, ear height and grain yield. The large environmental effects observed for grain yield and most traits in the inbred lines across varying environments indicated the variability of the inbred lines in response to the treatments imposed including herbicide application. Understanding these different responses is critical for identifying genotypes that not only perform well under controlled conditions but also exhibit resilience in environments that have varying conditions or management practices (Idehen *et al.*, 2023).

Broad-sense heritability (H^2) estimates varied widely across the environments, reflecting the influence of environmental conditions on the expression of most traits. Overall, heritability values ranged from low to moderatly high, suggesting that both genetic and environmental factors contributed to the observed phenotypic variation (Fontinele *et al.*, 2021; Kiani, 2017; Sheikh, 2019). Under the herbicide-stress environment, traits such as plant height, ear height, plant mortality and ear aspect showed relatively high heritability, implying strong genetic control

(Fontinele *et al.*, 2021). Conversely, grain yield exhibited low heritability, suggesting a major influence of the herbicide stress on yield performance. However, under optimal (manual weeding) environment, the heritability of grain yield increased to 59%, indicating that in the absence of stress, genetic variance contributed significantly to yield. Okunlola *et al.* (2023a) reported moderate to high heritability for grain yield on maize inbred lines, which signifies stability and reliability when selecting for yield traits across various environments. This result underscores that when environmental factors are minimized, genetic contributions become more pronounced. Adewale *et al.* (2020) also reported significant variation in grain yield among early maturing maize lines under both Striga-infested and Striga-free conditions, establishing the importance of genetic variance, particularly under optimal conditions devoid of stress. These findings are relevant as they highlight the heritability of grain yield, indicating that crops can potentially achieve their genetic potential when external factors are mitigated.

Across all environments combined, grain yield was positively correlated with plant height and ear height suggesting that taller plants with higher ears will have higher yields, but the strength of this relationship varied by environment (Yahaya *et al.*, 2021). Under drought environment, there was a strong positive correlation between grain yield and plant height suggesting that plant height may be indicative of drought tolerance. There was also a positive correlation between leaf chlorophyll content and ear height. This results supports the findings of (Ali *et al.*, 2017) and elucidates the underlying physiological mechanisms that confer drought tolerance while identifying superior traits for selecting promising candidates in developing drought-resistant varieties.

The high percentage of polymorphism observed in the study suggests a rich genetic diversity among the 50 inbred lines used in the study, which is vital for successful hybrid breeding

programme. In a study by (Muhammad *et al.*, 2017), it was found that 15 ISSR primers amplified 266 bands, with 88.9% being polymorphic, indicative of a strong genetic diversity among the maize genotypes. This result aligns with the findings of this current study, highlighting the use of ISSR markers in evaluating genetic variation. Also, the observed genetic diversity of 0.62 is similar to other studies (Soliman *et al.*, 2021) highlighting the effectiveness of ISSR markers in capturing genetic variability.

Among the ISSR primers used in this study, ISSR-06 was most informative marker, with the highest polymorphism information content (PIC) value of 0.78, which is indicative of the primer's effectiveness in distinguishing between genotypes. This is similar to a study by Amoon & Abdul-Hamed (2020) who in an ISSR-based diversity analysis of maize, noted that certain primers produced high percentages of polymorphic bands and high discriminatory power, thus serving as reliable tools for genetic diversity studies. This is corraborated by other studies which has shown that effective markers possess considerable abilites to reveal genetic variation (Feng & Noedoost, 2021; Ibrahim & Erdinc, 2020; Mowang *et al.*, 2021). In contrast, ISSR-05 had the lowest PIC value of 0.21 and fewest number of alleles which is indicative of the variability in marker performance and the significance of choosing the right primers for genetic studies. Such variability in the performance of different markers underscores the crucial importance of selecting appropriate primers for genetic studies. Prior research has highlighted that PIC values below 0.25 indicate very low genetic diversity, while values above this threshold suggest varying levels of genetic variability (Araújo *et al.*, 2023; Mowang *et al.*, 2021). Also, the inconsistency observed across different primers, such as the difference in PIC values between ISSR-06 and ISSR-05, is a common phenomenon reported in ISSR applications, where the varying ability of

primers to produce polymorphic bands significantly impacts the outcome of genetic diversity studies (Bahulikar *et al.*, 2023).

The dendrogram analysis grouped the 50 inbred lines intor four distinct clusters, with the predominant cluster comprising 47 inbred lines, while the remaining lines were distinctly separated. This pattern may be due to the limited level of polymorphism observed among the markers used. Out of the 30 primers screened, only 10 exhibited polymorphism, suggesting restricted genetic variability detectable by the selected markers. The number and degree of polymorphic markers play a crucial role in determining the resolution of genetic differentiation among inbred lines (Gedil *et al.*, 2023; Sin *et al.*, 2020). A higher number of polymorphic markers generally enhances the capacity to detect subtle genetic differences, leading to a more refined and distinct cluster formation. Studies have shown that a greater number of polymorphic loci corresponds to improved clustering precision and clearer genetic structure among genotypes. V. Kumar *et al.* (2017) reported low genetic similarity among maize inbred lines, implying a substantial level of polymorphism that favoured the clarity of clusters in their analysis. Similarly, Nehra *et al.* (2017), emphasized that polymorphic markers with high Polymorphic Information Content (PIC) values provide better discrimination between closely related genotypes. In the current study, the limited number of polymorphic primers likely reduced the overall PIC, limiting the ability to distinguish among genotypes and resulting in the observed dominance of a single large cluster.

Comparable findings were reported by Al-Hazemawi *et al.* (2024), who observed that higher polymorphism in SSR markers facilitated more refined classifications of maize lines. Also, Enoki *et al.* (2002) demonstrated that increasing the number of markers improves clustering precision and aligns clustering results more closely with the genetic distances among samples. These

observations collectively suggest that the limited number of polymorphic primers, 10 polymorphic primers out of a total of 30, may be insufficient for detailed genetic discrimination among the inbred lines assayed in this study. This suggests a need for further analysis employing additional or alternative markers to obtain a more comprehensive understanding of genetic diversity and improve clustering resolution.

3.7 Conclusion

The significant genetic variability among the inbred lines evaluated under optimum, drought and herbicide-treated environments observed indicate the presence of exploitable genetic diversity that can be harnessed for the improvement of stress tolerance and yield stability in maize breeding programmes.

The consistent performance of inbred lines such as C316-7, DHL 258 POP 2, and TZIL 25 (60E) 0.4 EMS across environments underscores their broad adaptability and resilience, highlighting their potential as elite parents for developing multiple stress-tolerant hybrids in breeding programmes.

Under herbicide stress, significant differences in mortality and tolerance levels were observed among inbred lines, confirming that response to nicosulfuron is genotype-dependent. Inbred lines such as C316-7, DHL 99 POP 2 and DHL 258 POP 2 exhibited high yield potential and low mortality, suggesting the presence of genes conferring herbicide tolerance. Conversely, several inbred lines exhibited severe phyotoxicity and complete yield loss, signifying genetic susceptibility. This study also highlighted the influence of herbicide timing and crop growth stage, with application at the V10 – VT stage being less injurious than early growth stage application.

Under drought conditions, inbred lines such as DHL 101 POP 5, EXP 124 and TZIL 25 (35G) 0.4 EMS demonstrated superior performance with high Yield Stability Index (YSI) and low Stress Susceptibility Index (SSI) values, confirming their potential for drought tolerance. Other genotypes, including C316-7, DHL 258 POP 2 and TZIL 25 (60E) 0.4 EMS, performed well

across all environments, indicating broad adaptability and the possession of favourable alleles for yield potential and multiple stress tolerance.

Molecular characterization using ISSR markers revealed significant genetic variation among the 50 inbred lines, confirming a broad genetic base. The high plymorphic percentage and the polymorphism information value of 0.78 for primer ISSR-06 demonstrated its high discriminatory power. Although clustering analysis grouped most inbreds into a predominant cluster, the presence of distinct minor clusters reflected genetic divergence, which can be harnessed to maximize heterosis in hybrid development.

CHAPTER FOUR

4.0 HYBRID PERFORMANCE UNDER VARYING ENVIRONMENTS

4.1 Introduction

Despite Ghana's agro-ecological advantage to contribute to high maize productivity through high incoming solar radiation and reduced incidence of pests and diseases due to prevailing low humidity and low night temperatures, the country continues to experience low yields on most smallholder farmer fields. This is attributed to a myriad of factors spanning from seed quality to the practice good agronomic practices. Continuous cropping on most soils, often with minimal soil fertility management, often leads to the depletion of organic matter and available nitrogen, both critical for maize productivity (Jahan *et al.*, 2020; Niu *et al.*, 2023; H. Zhou *et al.*, 2023). Additionally, the dependency on rainfall as the primary water source exacerbates yield variability, further limiting productivity.

One of the key strategies to address this is the development and adoption of resilient and stable maize hybrid varieties. This is essential to contribute to increased maize productivity and ensure food security especially in the wake of climate change. However, the productivity of a crop is not only influenced by its genetics but also by the environment, including climate variability and soil properties (Acquah & Kyei, 2012; Gyamerah *et al.*, 2024b). These factors are more likely to impact crop productivity, leading to lower yields on farmers' field compared to research stations (Hall *et al.*, 2024; MacCarthy *et al.*, 2018; Sileshi *et al.*, 2010; Van Ittersum *et al.*, 2016; Wangmo *et al.*, 2024). This "yield gap" highlights the need to evaluate developed maize genotypes across multiple environments before recommending for specific agro-ecological zones.

Identifying the best genotypes can be difficult due to the unpredictable effects of different environments on crop performance in unpredictable ways, a phenomenon known as genotype by

environment interaction (G x E interaction). This interaction reduces the stability of thebgenetic expression of agronomic traits thereby complicating the selection superior genotypes. Hence, breeding programmes must account for G x E interactions to improve selection efficiency and enhance the adaptability of developed genotypes across varying environments. Research by Boreddy *et al.* (2020a) highlights the complexities of G x E interactions, demonstrating that the stability and overall performance of maize hybrids can be inconsistent across various environments. In the study, specific hybrids were identified as stable and high-yielding across multiple locations, suggesting that both high mean performance and yield stability should be prioritized. In an analysis conducted by Getachew *et al.* (2021), it was emphasized that different genotypes exhibit varying responses to changing climatic and soil conditions. This variability is instrumental in defining the primary responsibility of maize breeders to select genotypes that demonstrate superior adaptability and stability across environments (Getachew *et al.*, 2021). Their findings reinforce the complexity of G x E interactions, which can lead to either significant advantages or disadvantages in specific contexts, complicating the selection of optimal cultivars.

The objectives of this study were to:

(i) identify superior maize hybrids with stable yield performance over the varying environments, and

(ii) examine how G x E interaction influenced yield and other agronomic traits.

4.2 Materials and Field experiments

4.2.1 Genetic materials

Twenty-four maize inbred lines were selected for this study (Table 4.1), comprising 12 white kernel inbred lines and 12 yellow kernel inbred lines. The inbred lines were selected based on their *'per se'* performance under optimum (manual weeding), herbicide-treated and drought environment as evaluated in Chapter three of this study.

The 24 inbred lines were used to generate ninety-six single cross hybrids using the North Carolina Design II (NCD II). To structure the mating scheme, the 12 white kernel lines were divided into three groups (A, B and C), each group containing 4 inbred lines each, and the 12 yellow kernel lines were also divided into three groups (C, D and E), also with each group containing 4 inbred lines each. Crosses were made between groups within each kernel colour type, where all inbred lines from one white group were crossed with all inbred lines from another white kernel group, and similarly for the yellow kernel groups.

Within these crosses, each inbred line in each group served as a female parent in one set of crosses and as a male parent in another set. This design was adopted to enable the estimation of maternal effects which provides key insights for selecting parents, improving hybrid performance and achieving consistent adaptation to environmental challenges (Brunel-Muguet *et al.*, 2025; John *et al.*, 2024; Rukundo *et al.*, 2017; X. Zhang *et al.*, 2016).

The planting of the inbred lines to generate 96 single-cross hybrids was carried out in 2023 at the WACCI Research Field, University of Ghana, Legon. The 96 single-cross hybrids plus four experimental checks were used in the study. The four experimental checks used in the study

included *Aburo Legon* (white kernel single-cross variety), *Obatanpa* (white kernel OPV), *Abontem* (yellow kernel OPV) and *Pioneer* (yellow kernel hybrid).

4.2.2 Field evaluation and data collection

A 10 x 10 (10 entries x 10 blocks) alpha lattice design with two replications was used in the study. The experiment consisted of single-row plots that were 4 m long, with a spacing of 0.75 m between adjacent rows and 0.40 m between plants within a row. Three seeds were planted per hill, and two weeks after emergence, the seedlings were thinned to two per hill resulting in a plant population density of approximately 66,666 plants per hectare.

The hybrid trials were evaluated at three locations under low soil nitrogen, induced drought, combined drought and low soil nitrogen and optimal environments for two years (Table 4.2). The management of the trials under drought and optimal environments as well as data collection relevant for this study were done according to the methods described in Chapter 3 of this thesis, section 3.2.4 to section 3.2.6. Leaf chlorophyll content (SPAD readings) was collected using the Cholorphyll metre, Konica Minolta SPAD-502 Plus, where measurements were taken from leaves of 10 random plants and the average data provided by the metre.

For maize production, low N soils are typically defined as soils whose available nitrogen is insufficient to meet the crop's nutrient demand for optimal growth and and yield. According to Landon (2014), soils with total nitrogen content below 0.15% are generally classified as low in nitrogen while those between 0.15-0.25% are considered as moderate.

Table 4. 1: Description of maize inbred lines selected for hybrid development.

S/N	INBRED	KERNEL COLOUR	SET	REACTION TO DT
1	C316-7	WHITE	A	S
2	TZIL 25 (40C) 0.4EMS	WHITE	A	S
3	DHL 186 POP 2	WHITE	A	S
4	TZIL 25 (43A) 0.4EMS	WHITE	A	T
5	TZIL 25 (60E) 0.4 EMS	WHITE	B	T
6	DHL 99 POP 2	WHITE	B	S
7	DHL 53 POP 4	WHITE	B	S
8	1368-150GY (119) S	WHITE	B	S
9	DHL 184 POP 2	WHITE	C	T
10	9006	WHITE	C	S
11	EXP 124	WHITE	C	T
12	1368	WHITE	C	S
13	HP020-5/2(A)/1/7	YELLOW	D	S
14	HP020-5/1(B)/1/7	YELLOW	D	S
15	LMI 135	YELLOW	D	T
16	LMI 102	YELLOW	D	S
17	LMI 140	YELLOW	E	T
18	LMI 141	YELLOW	E	S
19	HP020-4/1(A)/1/1	YELLOW	E	T
20	LMI 144	YELLOW	E	S
21	DHL 142 POP 1	YELLOW	F	S
22	HP020-6/2(9)/9/6	YELLOW	F	S
23	DHL 46 POP 1	YELLOW	F	S
24	DHL 18 POP 1	YELLOW	F	S

S = susceptibility; T = Tolerance; DT = Drought tolerance

Table 4. 2: Locations and treatments of hybrid trials evaluated over the past two years (2023-2024).

Treatment	Location	Region	Season	Month of planting	Year
Optimal	Legon	Greater Accra	Minor rainy season	October	2023
Optimal	Legon	Greater Accra	Major rainy season	May	2024
Optimal	Kwadaso	Ashanti	Major rainy season	May	2024
Drought	Legon	Greater Accra	Dry season	December	2023
Drought	Libga	Northern	Dry season	January	2024
Low soil N	Legon	Greater Accra	Major rainy season	April	2024
Low soil N	Kwadaso	Ashanti	Major rainy season	May	2024
DT + Low N	Legon	Greater Accra	Dry season	December	2023
DT + Low N	Libga	Northern	Dry season	January	2024

For trials evaluated under low soil N, soil samples were collected and analyzed to determine pH, nutrient levels and electrical conductivity (Table 4.3). The total nitrogen content across all blocks at the two locations, Legon and Kwadaso, ranged from 0.07 % to 0.28% and pH levels ranged between 4.9 and 6.5, indicating acidic soils with low nitrogen availability (Landon, 2014).

Based on the results, NPK fertilizer formulation was prepared to maintain the low-N environments using urea, single superphosphate and muriate of potash. The single superphosphate and the muriate of potash fertilizers were applied at the rate of 60 kg ha^{-1} each of phosphorous (P) and potassium (K). A split application of 30 kg ha^{-1} N was applied at 2 weeks after planting (WAP) and 4 WAP, at a rate of 15 kg ha^{-1} at each application respectively. This formulation ensured that nitrogen was the primary limiting factor, validating the site as a true low nitrogen environment.

Trials conducted at Libga under drought and combined drought and low soil N was inconclusive as the trial was destroyed by animals after the reproductive stage.

Table 4. 3: Soil chemical properties of experimental sites.

Description	Depth (cm)	pH (1:1)	EC (dS/m)	Organic C (%)	N (%)	Av. P (mg/kg)
Legon						
Block 1	0-30	5.6	0.19	1.04	0.28	86.92
Block 2	0-30	4.9	0.13	0.58	0.25	35.62
Block 3	0-30	5.3	0.1	0.61	0.26	44.06
Kwadaso						
Block 1	0-15	5.74	0.25	1	0.11	34.83
Block 2	15-30	5.28	0.3	0.72	0.1	27.86
Block 3	0-15	6.46	0.15	0.92	0.07	75.85
Block 4	15-30	6.54	0.17	1.08	0.09	96.75

It is important to note that not all the 96 hybrids generated were evaluated across all the test environments. Although pollination was successful during hybridization, limited seed set in some crosses meant that insufficient seed quantities were available for full replication across all environments. Owing to time constraints, these crosses could not be repeated to generate adequate seed for all locations. Also, in some locations, poor seed germination of certain hybrids resulted in missing plots. In other locations, stress conditions led to plant mortality which precluded yield data collection. Consequently, yield and related agronomic data were recorded only for hybrids that established and produced harvestable ears at each site.

4.3 Data analyses

4.3.1 Analyses of variance

Location and season combinations were considered as environments whereas the treatments (drought, low soil nitrogen, combined drought and low soil nitrogen, optimal and across environments) were regarded as management conditions. Separate ANOVA was performed for the single stress environments, optimal environments, and the combined stress environments. This was followed by combined ANOVA across environments. The ANOVA employed the mixed model in R using the *lme4* package (R Core Team, 2024). The environments, replications and blocks were treated as random factors while the hybrids were considered as fixed factors. Using the standard error of difference, means were separated. The statistical mixed model used was as follows:

$$Y_{ijkl} = \mu + H_i + E_j + R_{k(j)} + B_{l(jk)} + (H \times E)_{ij} + \varepsilon_{ijkl}$$

where: Y_{ijkl} was the observation for the i^{th} hybrid in the j^{th} environment, in the k^{th} replication (nested within environment), and in the l^{th} block (nested within replication and environment); μ is the overall mean; H_i is the fixed effect for the hybrid; E_j is the random effect for the environment; $R_{k(j)}$ is the random effect for the replications within each environment; $B_{l(jk)}$ is the random effect for the block within replications within environment; $(H \times E)_{ij}$ is the interaction between hybrid and environment, and ε_{ijkl} is the residual error term. Broad sense heritability of the measured traits was estimated using the restricted maximum likelihood (REML) in the *lmer* function from the *lme4* package.

Using the *VarCorr* function, the variance components were extracted and substituted into the heritability equation:

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_{GE}^2}{e} + \frac{\sigma_e^2}{r}}$$

where: H^2 is broad sense heritability; σ_G^2 is the genotypic variance; σ_{GE}^2 is the variance due to the hybrid-by-environment interactions; e is the number of environments; σ_e^2 is the residual variance and r is the number of replications.

4.3.2 Selection of tolerant hybrids using the base index (BSI)

A base index was used for selecting tolerant genotypes under drought, low N, combined drought and low N (Badu-Apraku *et al.*, 2011).

Under drought, a base index (BSI) incorporating yield (YLD), number of ears per plant (EPP), anthesis-silking interval (ASI), plant aspect (PASP), ear aspect (EASP) and leaf senescence (LS) was used to identify drought tolerant hybrids as follows:

$$BSI = [(2 \times YLD) + EPP - ASI - PASP - EASP - LS$$

A base index (BSI) incorporating YLD, EPP, ASI, PASP, EASP and stay-green characteristic (STGR) was used to identify low N tolerant lines as follows:

$$BSI = [(2 \times YLD) + EPP - ASI - PASP - EASP - STGR$$

To identify hybrids with combined tolerance to the combined environments, a base index incorporating YLD, EPP, ASI, PASP, EASP, LS and STGR was used as follows:

$$BSI = [(2 \times YLD) + EPP - ASI - PASP - EASP - LS - STGR$$

This BSI incorporated the BSI for the single stress environment, that is drought and low N, into one that could account for the combined stress environment.

Mean values for the measured traits were standardized before used to calculate the BSI as the traits were measured in different units. A positive BSI indicated tolerance whiles a negative BSI indicated susceptibility.

4.3.3 Genotype and Genotype by environment (GGE) biplot analysis

To examine the G x E interaction, the multivariate stability analysis was performed graphically based on GGE biplot using the GUI package of R studio developed by the R Core Team. The biplot was used to identify superior hybrids across drought, low N, combined drought and low N and optimal environments.

The biplot graph generated was based on multi-environment evaluation (*which-won-where*), genotype evaluation (*mean versus stability*) and tested environment ranking (*discriminative versus representative*).

4.4 Results

4.4.1 Variability for grain yield and other agronomic traits under varying environments

Analysis of variance revealed highly significant ($p < 0.001$) differences among sources of variation for most of the measured traits under drought environment (Table 4.4). The replication effect was highly significant for all traits, indicating the presence of environmental variation across replications. The effect of blocks within replications (REP:BLK) was also significant for most traits, suggesting that the experimental design effectively captured within-replication variability. Highly significant differences were observed among the female parental lines for all traits except cholophyll content (SPAD). Similarly, male parental lines differed significantly ($p < 0.01$ or $p < 0.05$) for almost all the traits except grain yield (YLD), ear aspect (EASP), plant aspect (PASP), and SPAD. The female x male interaction, which represents specific combining ability (SCA) was significant ($p < 0.05$) for all traits except plant height (PH), EASP, PASP, leaf senescence (LS) and SPAD, indicating that these traits were predominantly controlled by additive rather than dominance effects. Broad-sense heritability (H^2) estimates ranged from 0.09 (SPAD) to 0.82 (DYANTH) while narrow-sense heritablity (h^2) were generally low to moderate, ranging from 0.01 (EASP) to 0.26 (DYANTH).

The combined analysis of variance across LN environments revealed highly significant ($p < 0.001$) differences among environments for all measured traits (Table 4.5). This indicates that the testing environments at Legon and Kwadaso were distinct and imposed varying degrees of stress, leading to differences in hybrid performance. Significant differences among female and male parental lines were observed for most traits, suggesting genetic variability among the parental lines. Female effects were highly significant for YLD, days to 50% anthesis (DYANTH), days to 50% silking (DYSK), PH, PASP, 100 seed kernel weight (100-KW) and

LS, whereas non-significant differences was recorded for anthesis-silking interval (ASI) and SPAD readings. Similarly, the male main effects were significant ($p < 0.001$ or $p < 0.05$) for all traits except ASI and SPAD. The female x male interaction was significant for most traits except for ASI, EH and SPAD. The environment x genotype interactions (ENV x FEMALE, ENV x MALE and ENV x HYB) were significant for most traits except ASI and SPAD, implying that the performance of hybrids and parental lines were not consistent across environments. However, the ENV x FEMALE x MALE interaction was not significant for any trait, indicating that the rank order of specific hybrid combinations was largely stable across environments. Broad sense heritability (H^2) estimates were moderate to high for most traits, ranging from 0.25 (SPAD) to 0.61 (YLD, DYANTH and DYSK) while narrow sense heritability (h^2) estimates were generally low ranging from 0.02 (100-KW) to 0.23 (DYANTH).

Table 4. 4: Mean squares and heritability estimates of grain yield and other traits of hybrids evaluated in drought (DT) environment at Legon (dry season) in 2023.

Source	Df	YLD	DYANTH	DYSK	ASI	PH	EH	EASP	PASP	100-KW	LS	SPAD
REP	1	10034946.04**	331.03**	131.04**	45.52**	11370.83**	4694.57**	5.17*	3.89*	141.46**	112.64**	1307.64**
REP:BLK	18	1898833.43**	20.43**	29.57**	9.76**	1367.80**	637.23**	1.34*	1.06ns	28.25**	5.55**	78.44ns
FEMALE	23	1547510.34**	27.92**	49.61**	16.61**	1130.94**	474.84**	1.15*	1.72**	38.93**	2.86*	115.20ns
MALE	18	1049608.05ns	24.51**	34.42**	8.03*	765.86*	382.92**	0.46ns	0.91ns	35.97**	2.60*	70.78ns
HYB	86	1348129.67**	17.88**	30.51**	9.65**	784.77**	330.15**	1.00*	1.23**	26.19**	2.46*	79.70ns
FEMALE: MALE	45	1051252.96*	6.08**	15.30*	6.91*	417.11ns	167.95*	0.95ns	0.91ns	14.04*	1.68ns	56.71ns
Residuals	68	608075.86	2.36	6.73	3.83	403.46	87.94	0.63	0.66	7.47	1.34	68.30
Grand mean		1687.14	52.09	55.6	3.51	195.59	82.06	3.01	3.14	22.51	3.05	41.89
H^2		0.61	0.82	0.76	0.65	0.23	0.70	0.51	0.47	0.70	0.39	0.09
h^2		0.03	0.26	0.16	0.09	0.18	0.15	0.01	0.06	0.16	0.08	0.09

YLD, grain yield (kg/ha); DYANTH, days to 50% anthesis; DYSK, days to 50% silking; ASI, anthesis-silking interval; PH, plant height (cm); EH, ear height (cm); PASP, Plant aspect; LS, leaf senescence; SPAD, leaf chlorophyll content; STGR, stay-green characteristic; H^2, broad-sense heritability; h^2, narrow-sense heritability.

**=significant at 0.05 probability level, **=highly significant at 0.001 level, ns = not significant*

Table 4. 5: Mean squares, variance and heritability estimates of grain yield and other traits of hybrids evaluated across LN environments at Legon and Kwadaso in 2023 and 2024.

Source	Df	YLD	DYANTH	DYSK	ASI	PH	EH	PASP	100-KW	LS	SPAD
ENV	1	121417013.26**	3144.05**	4145.64**	69.14**	684571.65**	45887.77**	147.68**	602.73**	452.28**	12925.25**
FEMALE	23	1019242.01**	40.18**	56.01**	7.42ns	1428.59**	632.30**	1.10**	86.33*	2.26*	86.49ns
MALE	18	710465.00*	27.09**	37.91**	7.27ns	1502.41**	486.15**	0.95*	91.42*	3.05**	72.45ns
FEMALE:MALE	46	626832.46*	9.51*	17.54*	5.49ns	701.25**	221.98ns	0.67*	85.68**	2.40**	62.50ns
HYB	87	747876.20**	21.26**	31.92**	6.37ns	1059.29**	385.11**	0.84**	87.04**	2.50**	70.90ns
ENV:FEMALE	23	708263.10*	10.03*	19.36*	6.82ns	1039.78**	341.96**	0.72*	100.44*	1.72ns	61.59ns
ENV:MALE	18	808588.52*	10.90*	24.37*	4.84ns	884.94**	330.46*	1.11**	67.21ns	1.90ns	67.72ns
ENV:HYB	87	619758.71*	8.54**	15.93*	5.06ns	950.28**	324.08**	0.72**	75.07**	2.13**	57.66ns
ENV:REP	2	1519000.66*	69.21**	76.15**	13.10ns	10562.35**	2770.66**	4.23**	101.20ns	3.12ns	181.85ns
ENV:REP:BLK	36	1331032.84**	13.66**	17.23*	5.88ns	1576.51**	566.36**	0.85**	53.17ns	4.02**	115.14*
ENV:FEMALE:MALE	46	308148.01ns	6.26ns	10.10ns	4.29ns	545.17ns	170.29ns	0.34ns	63.48ns	1.65ns	52.97ns
Residuals	138	388204.97	5.33	11.34	4.97	386.53	156.45	0.44	44.92	1.21	64.18
Mean		1016.62	57.79	61.81	4.02	167.57	68.89	3.59	26.35	3.28	3.14
H^2		0.61	0.61	0.61	0.34	0.40	0.44	0.59	0.41	0.50	0.25
h^2		0.03	0.23	0.14	0.03	0.08	0.12	0.03	0.02	0.04	0.03

YLD, grain yield (kg/ha); DYANTH, days to 50% anthesis; DYSK, days to 50% silking; ASI, anthesis-silking interval; PH, plant height (cm); EH, ear height (cm);; PASP, Plant aspect; LS, leaf senescence; SPAD, leaf chlorophyll content; STGR, stay-green characteristic; H^2, broad-sense heritability; h^2, narrow-sense heritability.

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**=significant at 0.05 probability level, **=highly significant at 0.001 level, ns = not significant.*

Under combined drought and low soil N (DTLN) environment, there was significant differences ($p < 0.001$ or $p < 0.05$) among sources of variation for all traits studied (Table 4.6). Replication effects were higly significant for all traits except ASI and STGR, indicating appreciable environmental variation across replications. Significant differences among female parental lines were detected for all measured traits except STGR. Similarly, the male parental lines also differed siginificant for most traits, including DYANTH, DYSK, ASI, PH, EH, EASP, 100-KW, LS and SPAD readings. The female x male interaction (FEMALE:MALE), representing SCA effects, was significant for ASI, PH, EH, EASP, PASP, 100-K1, SPAD and STGR. Non-significant SCA effects was observed for grain yield and flowering traits indicating that yield variation was mainly governed by additive rather than dominance effects in this environment. Broad-sense heritability (H^2) estimates ranged from 0.53 (STGR) to 0.79 (PH), reflecting moderate to high genetic control across traits. Narrow-sense heritability (h^2) estimates were generally low to moderate, ranging from 0 (STGR) to 0.39 (DYANTH).

Across optimal environments, the combined analysis of variance revealed highly significant ($p < 0.001$) differences among environments for almost all measured traits except 100-KW, indicating substantial environmental variability across locations (Table 4.7). Significant differences among female and male parental lines were also detected for all traits. Similarly, the SCA effects was highly significant for all traits. The presence of significant environment x genotype (ENX x FEMALE, ENV x MALE and ENV x HYB) interactions for most traits, particularly YLD, DYANTH, DYSK and PH, indicates that the performance of hynbrids and parental lines was not consistent across environments. Conversely, the ENV x FEMALE x MALE interaction was not significant for most traits, implying that the relative ranking of hybrid combinations was generally stable across locations.

Table 4. 6: Mean squares and heritability estimates of grain yield and other traits of hybrids evaluated in combined drought and low soil N (DTLN) environment at Legon (dry season) in 2023.

Source	Df	YLD	DYANTH	DYSK	ASI	PH	EH	EASP	PASP	100-KW	LS	SPAD	STGR
REP	1	3345799.11**	111.36**	91.64**	0.96ns	2331.64**	2405.74**	8.64**	2.05*	68.75*	9.55**	760.53**	0.01ns
FEMALE	23	540802.30*	36.89**	56.41**	14.30**	1482.57**	619.18**	0.97*	1.21**	40.01**	2.94**	30.81**	0.79ns
MALE	18	517227.33*	31.11**	59.91**	14.67**	765.14**	356.64**	1.10*	0.97*	24.36*	3.52**	36.83**	0.54ns
HYB	87	551733.75**	19.81**	35.32**	11.96**	786.93**	286.98**	1.02**	0.90**	30.62**	2.48**	30.82**	0.78*
REP:BLK	18	678671.34**	7.81ns	17.12ns	11.74**	334.03*	143.83**	1.13*	0.57ns	29.60*	5.67**	51.97**	1.12*
FEMALE:MALE	46	430256.16ns	6.23ns	12.73ns	7.99*	372.72**	80.63*	0.95*	0.68*	23.20*	1.04ns	22.79*	0.76*
Residuals	69	244880.22	4.99	10.29	4.40	152.95	48.04	0.54	0.43	12.50	0.69	12.80	0.49
Mean		999.19	54.57	59.69	5.13	177.69	67.99	3.51	3.43	21.78	3.71	30.45	3.02
H^2		0.61	0.59	0.55	0.65	0.79	0.74	0.60	0.57	0.65	0.60	0.63	0.53
h^2		0.03	0.39	0.33	0.09	0.18	0.38	0.01	0.07	0.05	0.21	0.05	0.00

YLD, grain yield (kg/ha); DYANTH, days to 50% anthesis; DYSK, days to 50% silking; ASI, anthesis-silking interval; PH, plant height (cm); EH, ear height (cm);; PASP, Plant aspect; LS, leaf senescence; SPAD, leaf chlorophyll content; STGR, stay-green characteristic; H^2, broad-sense heritability; h^2, narrow-sense heritability.

**=significant at 0.05 probability level, **=highly significant at 0.001 level, ns = not significant.*

Table 4. 7: Mean squares and heritability estimates of grain yield and other traits of hybrids evaluated across optimal environments at Legon and Kwadaso in 2023 and 2024.

Source	Df	YLD	DYANTH	DYSK	ASI	PH	EH	EASP	PASP	100-KW	SPAD
ENV	2	6700647.85**	620.33**	961.95**	52.98**	2004128.03**	519473.10**	8.88**	103545.31**	36.21ns	36735.16**
FEMALE	23	2295701.72**	48.53**	51.06**	3.21*	5328.60**	1682.50**	1.24*	164.62**	40.69**	314.01**
MALE	18	2858985.66**	19.83**	23.07**	3.52*	3149.23**	999.35**	1.21*	81.19**	59.67**	398.46**
HYB	93	2347051.07**	23.72**	29.77**	3.57**	3685.68**	1083.83**	1.15**	107.13**	37.94**	350.49**
ENV:REP	3	4216125.31*	34.88**	25.44*	1.00ns	315.00ns	1959.70**	3.93**	73.57**	19.35ns	2386.12**
FEMALE:MALE	52	2192972.88**	14.25**	22.83**	3.75**	3143.46**	849.38**	1.09**	90.68**	29.26**	350.99**
ENV:FEMALE	46	1408620.11ns	15.27**	18.35**	2.15ns	3191.14**	1016.47**	0.74ns	161.55**	16.53ns	262.26**
ENV:MALE	36	1741034.43*	16.81**	17.78**	2.43ns	1692.58**	1020.96**	0.70ns	80.96**	21.24*	306.13**
ENV:HYB	186	1467658.70*	12.47**	15.51**	2.18*	2141.99**	861.59**	1.41**	105.17**	14.78ns	228.50**
ENV:REP:BLK	54	2222973.95**	16.12**	20.81**	1.92ns	917.76**	1345.97**	1.61**	70.72**	13.69ns	349.71**
ENV:FEMALE:MALE	104	1205026.25ns	8.17*	11.24*	1.91ns	1634.36**	520.34**	0.72ns	71.43**	12.05ns	147.33ns
Residuals	225	1067238.78	5.47	7.88	1.71	310.26	266.38	0.63	8.82	14.44	116.67
Mean		2982.97	54.71	56.49	1.78	137.42	104.12	3.31	16.34	28.29	63.88
H^2		0.63	0.64	0.69	0.67	0.66	0.56	0.53	0.34	0.72	0.73
h^2		0.05	0.14	0.09	0.03	0.06	0.05	0.06	0.00	0.09	0.02

YLD, grain yield (kg/ha); DYANTH, days to 50% anthesis; DYSK, days to 50% silking; ASI, anthesis-silking interval; PH, plant height (cm); EH, ear height (cm); 100-KW, 100-grain kernel weight (g); PASP, Plant aspect; SPAD, leaf chlorophyll content; H^2, broad-sense heritability; h^2, narrow-sense heritability .

**=significant at 0.05 probability level, **=highly significant at 0.001 level, ns = not significant.*

Broad-sense heritability (H^2) estimates ranged from 0.34 (PASP) to 0.73 (SPAD), indicating moderate to high genetic control across traits. Narrow-sense heritability (h^2) estimates were generally low, ranging from 0 (PASP) to 0.14 (DYANTH).

The combined analysis of variance across all environments revealed highly significant differences among environments for all measured traits (Table 4.8). This confirms the presence of strong environmental heterogeneity which influenced hybrid performance. Highly significant ($p < 0.001$) differences were detected among female and male parents for all traits. These results indicate substantial additive genetic variability among the parental lines, suggesting the presence of exploitable genetic diversity for yield and other agronomic traits across environments. The SCA effects was highly significant for all traits highlighting the potential for heterotic responses among specific hybrid combinations. Significant environment x genotype (ENV x FEMALE, ENV x MALE and ENV x HYB) interactions were observed almost all the traits, indicating that the relative performance of genotypes varied across environments. This reflects a strong genotype-by-environment interaction emphasizing the need to identify stable and high-yielding hybrids across different stress environments. Broad-sense heritability (H^2) estimates ranged from 0.56 (SPAD) to 0.89 (PH), indicating moderate to high genetic control across traits. Narrow-sense heritability (h^2) estimates were generally low, ranging from 0.03 (PASP) to 0.32 (DYANTH).

Table 4. 8: Pooled mean squares, variance and heritability estimates of grain yield and other traits of hybrids evaluated across optimum, low soil N, drought and combined DTLN environments at Legon and Kwadaso in 2023 and 2024.

Source	Df	YLD	DYANTH	DYSK	ASI	PH	EH	EASP	PASP	100-KW	SPAD
ENV	6	202182047.96**	1438.23**	2306.49**	367.25**	879264.12**	238780.14**	42.52**	43223.70**	1470.49**	63065.39**
FEMALE	23	2671294.91**	124.69**	150.51**	16.52**	5544.76**	2568.44**	1.47**	86.35**	86.28**	221.60**
MALE	18	2532491.17**	72.79**	98.64**	15.95**	3663.21**	1550.15**	1.72**	60.76**	125.86**	204.82**
HYB	93	2318523**	55.74**	76.25**	11.90**	4509.51**	1282.34**	1.46**	91.34**	82.36**	189.74**
ENV:REP	7	4129896.88**	98.77**	65.24**	10.80**	5107.93**	2658.60**	4.17**	33.51**	62.98*	1371.63**
FEMALE:MALE	52	2084855.54**	19.32**	35.68**	8.45**	4344.84**	620.42**	1.36**	104.14**	65.65**	170.74**
ENV:FEMALE	138	1049286.78**	11.15**	18.20**	5.46**	1495.69**	516.37**	0.83**	57.44**	40.59**	146.67**
ENV:MALE	108	970016.09*	12.31**	18.95**	4.40*	1018.36**	477.17**	0.65ns	30.42**	31.51*	166.73**
ENV:HYB	529	1036050.54**	9.70**	15.71**	4.69**	1173.82**	476.88**	0.81**	40.34**	32.60**	145.13**
ENV:REP:BLK	126	1776849.54**	14.87**	20.64**	5.65**	1167.38**	926.10**	1.55**	32.37**	30.39*	229.65**
ENV:FEMALE:MALE	283	853399.90*	6.66**	11.42*	4.11*	887.08**	312.28**	0.74*	28.71**	27.64*	106.18*
Residuals	504	715422.65	4.99	9.06	3.24	327.87	186.78	0.57	4.22	22.02	83.92
Grand mean		1986.00	55.19	58.29	3.11	159.39	86.27	3.49	9.19	26.06	47.36
H^2		0.76	0.85	0.85	0.72	0.89	0.73	0.65	0.84	0.75	0.56
h^2		0.07	0.32	0.23	0.13	0.07	0.22	0.07	0.03	0.10	0.04

YLD, grain yield (kg/ha); DYANTH, days to 50% anthesis; DYSK, days to 50% silking; ASI, anthesis-silking interval; PH, plant height (cm); EH, ear height (cm);; PASP, Plant aspect; 100-grain kernel weight (g); SPAD, leaf chlorophyll content, $\sigma^2 g$, genotypic variance; H^2, broad-sense heritability; h^2, narrow-sense heritability.

**=significant at 0.05 probability level, **=highly significant at 0.001 level, ns = not significant*

4.4.2 Performance of single-cross hybrids under drought, low N, combined drought and low N, optimal and across environments

Using the multiple traits selection index (BSI), HP020-4/1(A)/1/1 X DHL 46 POP 1, HP020-4/1(A)/1/1 X DHL 18 POP 1 and HP020-6/2(9)/9/6 X LMI 135 were identified as the top three superior hybrids under drought environment (Table 4.9). Eleven (11) out of the top fifteen (15) best performing genotypes had at least one (1) drought tolerant parental inbred line while six (6) out of the ten (10) least performing genotypes had both parental lines being susceptible to drought. The highest yielding hybrid, HP020-4/1(A)/1/1 X DHL 46 POP 1, out-performed the best check, Aburo Legon, by 41%. Under drought environment, the mean grain yield was 1651.40 kg/ha with a mean yield percentage loss of 45%. Anthesis-silking interval ranged from -2 days to 10 days.

Across LN environments, based on the BSI, LMI 102 X HP020-4/1(A)/1/1, LMI 102 X LMI 140 and DHL 99 POP 2 X DHL 184 POP 2 were the the top three superior hybrids (Table 4.10). Comparatively, leaf chlorophyll content (SPAD) was the lowest under LN environment recording an average of 32 across all hybrids evaluated. The highest performing hybrid, LMI 102 X LMI 140, outyielded the best check, Pioneer by approximately 7%. The mean grain yield under low N was 1030 kg/ha with a mean yield percentage loss of 64.35% (Table 4.7). The mean anthesis-silking interval across all hybrids was 4 days.

LMI 144 X DHL 46 POP 1, 1368-150GY (119)S X 9006 and HP020-5/1(B)/1/7 X LMI 140 were the top three superior hybrids based on their BSI values under combined drought and LN environment (Table 4.11). DHL 99 POP 2 X 1368, the highest yielding hybrid, out-performed two checks, Abontem and Obatanpa by 40% and 74% respectively.

Table 4. 9: Top 15 and bottom 10, hybrids, including 4 checks, selected based on grain yield and other traits under drought environment in 2023.

HYBRID	YLD	ASI	PASP	LS	SPAD	PH	EH	EASP	YPN	BSI
HP020-4/1(A)/1/1 X DHL 46 POP 1	3802.38	1	2	2	49	222	94	2	4.27	11.31
DHL 142 POP 1 X LMI 135	3336.60	3	3	2	54	197	83	3	1.51	6.58
TZIL 25 (40C) 0.4 EMS X DHL 99 POP 2	3023.58	2	2	1	46	202	92	2	21.33	7.62
HP020-6/2(9)/9/6 X LMI 135	2878.51	3	2	1	42	206	82	2	5.41	8.15
EXP 124 X DHL 186 POP 2	2832.90	0	2	2	49	208	94	3	4.26	6.74
HP020-4/1(A)/1/1 X DHL 18 POP 1	2775.24	2	2	2	44	182	73	2	9.32	8.27
DHL 46 POP 1 X HP020-5/2(A)/1/7	2762.77	2	2	2	49	226	101	3	5.55	5.76
DHL 184 POP 2 X TZIL 25 (43A) 0.4 EMS	2725.71	2	3	2	45	191	71	3	9.27	4.67
HP020-5/1(B)/1/7 X LMI 140	2723.06	2	3	3	52	242	118	3	21.07	4.70
LMI 135 X LMI 140	2696.75	2	3	2	43	204	85	3	36.88	4.42
LMI 140 X DHL 142 POP 1	2570.79	3	3	2	53	228	76	3	21.01	3.84
LMI 102 X LMI 141	2550.51	3	2	2	46	224	99	3	10.80	3.89
HP020-5/2(A)/1/7 X HP020-4/1(A)/1/1	2532.12	1	3	3	36	215	94	3	4.59	4.92
DHL 184 POP 2 X TZIL 25 (40C) 0.4 EMS	2501.39	1	2	1	45	204	81	2	25.63	7.94
LMI 102 X LMI 144	2500.89	5	3	3	49	217	88	3	6.72	5.46
LMI 144 X DHL 18 POP 1	678.64	6	4	5	52	166	82	3	68.09	-5.80
C316-7 X 1368-150GY (119)S	675.54	4	4	3	45	160	55	4	71.88	-4.64
LMI 141 X HP020-6/2(9)/9/6	673.11	10	5	5	32	192	82	4	78.39	-8.81
TZIL 25 (40C) 0.4 EMS X 1368-150GY (119)S	437.95	5	5	5	42	141	54	4	83.15	-8.07
9006 X TZIL 25 (40C) 0.4 EMS	365.43	3	4	6	45	183	74	4	84.03	-9.05
TZIL 25 (40C) 0.4 EMS X TZIL 25 (60E) 0.4 EMS	363.62	7	5	6	44	173	68	4	74.62	-9.43
C316-7 X DHL 53 POP 4	322.40	8	5	5	29	174	73	5	89.13	-12.03
DHL 53 POP 4 X EXP 124	321.18	9	4	3	31	194	89	4	89.91	-9.58
TZIL 25 (43A) 0.4 EMS X TZIL 25 (60E) 0.4 EMS	207.41	6	5	4	35	167	66	5	92.38	-9.92
1368 X TZIL 25 (43A) 0.4 EMS	186.36	2	5	3	34	189	82	5	92.75	-5.92
ABURO LEGON-CHK 1	2235.80	2	2	3	36	191	82	3	32.85	4.56
OBATANPA-CHK 2	1086.15	6	4	4	33	205	97	4	43.56	-2.56
ABONTEM-CHK 3	1667.57	-1	3	4	34	212	79	3	45.25	0.17
PIONEER-CHK 4	298.04	2	4	2	45	191	89	5	90.68	-7.64
GRAND MEAN	**1651.40**	**3**	**3**	**3**	**42**	**196**	**82**	**3**	**45.09**	
SE	**82.83**	**0.23**	**0.08**	**0.12**	**0.67**	**2.04**	**1.33**	**0.08**		

YLD, grain yield (kg/ha); ASI, anthesis-silking interval; ASI, anthesis-silking interval; LS, leaf senescence; PASP, plant aspect; PH, plant height (cm);EH, ear height (cm); SPAD, leaf chlorophyll content; EASP, ear aspect; YPN, yield penalty; BSI, base selection index; SE, standard error.

Table 4. 10: Top 15 and bottom 10 hybrids, including 4 checks, selected based on grain yield and other traits across LN environments in 2024.

HYBRID	YLD	ASI	PASP	SPAD	LS	SG	PH	EH	RL	SL	100KW	EASP	YPN	BSI
LMI 102 X LMI 140	2200	3	3	46	3	3	183	78	3	4	30.5	3	43.94	9.99
LMI 102 X HP020-4/1(A)/1/1	1937	3	3	41	4	3	204	86	3	3	30.25	3	46.82	11.18
DHL 186 POP 2 X TZIL 25 (60E) 0.4 EMS	1801	3	3	39	2	3	172	71	3	4	21.25	3	48.56	8.61
DHL 46 POP 1 X LMI 102	1750	4	3	36	3	3	201	87	2	2	31.75	3	23.67	8.18
DHL 186 POP 2 X 1368-150GY (119)S	1747	5	3	38	4	3	190	79	2	2	30.25	4	42.58	5.42
DHL 99 POP 2 X DHL 184 POP 2	1720	2	3	31	4	3	174	75	2	2	29	4	38.71	9.09
DHL 99 POP 2 X 1368	1679	3	3	32	3	3	193	81	2	2	30.5	3	37.02	7.48
HP020-5/1(B)/1/7 X HP020-4/1(A)/1/1	1654	2	3	26	4	3	187	71	3	3	29	4	26.53	6.63
C316-7 X DHL 99 POP 2	1590	4	4	28	5	4	167	75	3	3	26	4	52.06	0.80
9006 X DHL 186 POP 2	1570	2	3	36	4	3	187	79	2	3	29.25	3	40.87	7.66
LMI 140 X DHL 18 POP 1	1503	3	4	34	3	3	173	76	2	3	27.75	4	61.16	2.53
DHL 186 POP 2 X DHL 99 POP 2	1467	3	3	43	3	3	173	76	2	2	30.75	3	21.00	6.53
HP020-5/2(A)/1/7 X LMI 140	1463	4	3	28	4	3	188	85	2	2	27	3	34.93	5.07
DHL 99 POP 2 X 9006	1456	3	4	34	5	3	153	57	3	3	26.5	3	66.77	3.84
1368 X TZIL 25 (40C) 0.4 EMS	1434	2	4	29	2	3	163	72	4	5	28.75	4	57.48	3.16
TZIL 25 (40C) 0.4 EMS X TZIL 25 (60E) 0.4 EMS	477	5	5	30	4	4	128	47	2	2	28.5	5	66.67	-8.34
DHL 53 POP 4 X DHL 184 POP 2	420	6	4	28	3	4	150	61	3	3	15	5	79.56	-6.83
HP020-4/1(A)/1/1 X HP020-6/2(9)/9/6	419	3	4	31	4	4	156	56	3	3	15.25	5	78.18	-6.67
HP020-6/2(9)/9/6 X HP020-5/2(A)/1/7	402	7	5	29	3	4	165	59	2	2	27.5	5	87.32	-6.92
9006 X TZIL 25 (40C) 0.4 EMS	392	4	4	27	5	4	191	65	2	3	13.5	4	82.86	-6.95
1368-150GY (119)S X 1368	381	7	4	34	4	4	156	60	3	2	20	5	87.96	-9.00
DHL 18 POP 1 X HP020-5/1(B)/1/7	273	6	4	32	4	4	147	58	2	3	23.5	5	92.56	-9.99
1368 X C316-7	271	5	4	33	4	4	172	56	2	3	14	4	86.54	-8.93
TZIL 25 (40C) 0.4 EMS X DHL 53 POP 4	243	3	4	29	3	2	169	68	3	3	19.5	4	91.99	-2.68
LMI 141 X HP020-6/2(9)/9/6	198	7	4	31	3	4	154	56	2	2	21.75	5	93.66	-12.91
ABURO LEGON-CHK 1	1409	4	3	31	4	3	172	69	3	2	21.75	4	57.69	4.08
OBATANPA-CHK 2	833	5	4	30	4	3	179	82	2	3	31	4	56.71	-2.05
ABONTEM-CHK 3	1122	3	4	39	4	3	170	66	2	3	24.75	4	63.15	0.73
PIONEER-CHK 4	2055	3	3	34	3	2	183	80	2	2	31	3	35.73	12.50
GRAND MEAN	**1030**	**4**	**4**	**32**	**3**	**3**	**168**	**69**	**2**	**3**	**26.41**	**4**	**64.35**	
SE	**45.29**	**0.13**	**0.05**	**0.44**	**0.08**	**0.04**	**1.67**	**1.01**	**0.05**	**0.07**	**0.48**	**0.05**	**1.71**	

YLD, grain yield (kg/ha); ASI, anthesis-silking interval; ASI, anthesis-silking interval; LS, leaf senescence; PASP, plant aspect; PH, plant height (cm);EH, ear height (cm); SPAD, leaf chlorophyll content; EASP, ear aspect; SG, stay-green characteristic; 100KW, 100 kernel weight (g);RL, root lodging; SL, stalk lodging; YPN, yield penalty; BSI, base selection index; SE, standard error.

Notably, under combined stress environment, Aburo Legon and Pioneer, which were used as checks in the study, had the highest BSI values and recorded the highest grain yield of 2679 kg/ha and 2564 kg/ha respectively. Out of the top fifteen (15) best performing hybrids, only three (3) had at least one drought tolerant parent with the remaining eleven (11) having both parents being susceptible to drought. The mean grain yield under the combined drought and low N environment was 1040 kg/ha with a yield percentage loss of 64.51%. Anthesis-silking interval ranged from 1 to 13 days with a mean of 5 days. The ten (10) least performing hybrids also had the highest anthesis-silking interval in comparison to the top fifteen (15) best performing hybrids. Leaf senescence ranged from 2 to 7 with the worst performing hybrid, TZIL 25 (60E) 0.4 EMS X 9006, of grain yield 36 kg/ha scoring the highest of 7.

Across optimum environments, the grain yield ranged from 1432 kg/ha to 4540 kg/ha with a mean grain yield of 2979 kg/ha (Table 4.12). 1368 X DHL 186 POP 2, DHL 99 POP 2 X 9006 and LMI 135 X LMI 140 were the three highest yielding hybrids out-performing Aburo Legon by 27%, 24% and 22% respectively. Anthesis-silking interval ranged from 0 to 4 days and recorded a mean of 2 days.

DHL 53 POP 4 X 1368, LMI 135 X HP020-4/1(A)/1/1 and LMI 102 X LMI 140 were the highest yielding hybrids across all environments out-performing Aburo Legon by 25%, 17% and 13% respectively. Across all environments, a grand mean yield of 1986 kg/ha was recorded with a mean anthesis-silking interval of 3 days (Table 4.13).

The performance of the hybrids varied across the varying environments with significant fluctuations in performance under drought and low N environment (Figure 4.1). Hybrids such as HP020-4/1(A)/1/1 X DHL 46 POP 1 (G-073) performed well under drought but poorly under low N

environment. 1368 X DHL 186 POP 2 (G-004) which was the best performer across optimum environments performed poorly under the stress environments. Pioneer (G-020), one of the checks used in the study, performed better under combined drought and low N and under the single stress low N environment as compared to the single stress drought environment where it performed poorly. Aburo Legon (G-097), a check used in the study, was the highest yielding under combined drought and low N while under the single stress environment of drought and low N, it performed averagely though comparatively it performed better under drought than under low N environment.

Table 4. 11: Top 15 and bottom 10 hybrids, including 4 checks, selected based on grain yield and other traits under combined drought and low soil N environment in 2023.

HYBRID	YLD	ASI	PASP	LS	STGR	PH	EH	RL	SL	100KW	EASP	YPN	BSI
DHL 99 POP 2 X 1368	2333	3	3	5	2	178	67	1	1	25.50	3	12.48	8.18
1368-150GY (119)S X 9006	2091	2	3	3	2	181	64	1	1	28.00	3	5.04	9.67
LMI 144 X DHL 18 POP 1	2024	3	3	4	3	180	80	1	2	17.00	3	4.84	5.26
LMI 144 X DHL 46 POP 1	1914	1	2	3	2	200	85	1	1	23.50	3	38.46	10.67
DHL 186 POP 2 X 1368-150GY (119)S	1829	4	3	4	3	214	89	1	3	29.00	3	39.89	5.99
LMI 102 X LMI 140	1806	5	3	4	3	222	92	2	1	25.00	3	53.97	7.04
TZIL 25 (40C) 0.4 EMS X DHL 99 POP 2	1806	3	4	3	3	170	62	1	1	27.00	2	40.26	4.65
DHL 142 POP 1 x HP020-5/2(A)/1/7	1794	4	3	2	3	178	83	1	4	18.00	4	33.55	4.50
HP020-5/1(B)/1/7 X LMI 140	1785	3	2	3	2	204	83	1	3	19.50	3	48.26	9.03
DHL 46 POP 1 X HP020-5/2(A)/1/7	1723	3	3	3	3	197	89	1	1	17.50	4	37.62	5.20
9006 X C316-7	1695	4	3	2	2	167	53	1	2	28.50	3	41.06	8.14
C316-7 X DHL 53 POP 4	1687	5	4	5	3	185	70	1	1	26.50	3	43.09	2.50
DHL 18 POP 1 X LMI 135	1655	4	3	3	2	177	73	1	1	20.00	3	50.02	8.04
DHL 18 POP 1 X HP020-5/2(A)/1/7	1640	3	3	4	3	182	81	1	1	16.50	2	36.97	5.54
DHL 184 POP 2 X DHL 186 POP 2	1626	4	4	2	4	176	56	1	1	24.00	4	33.62	2.63
DHL 53 POP 4 X DHL 184 POP 2	334	8	4	2	4	167	63	1	1	25.00	4	83.74	-5.13
9006 X TZIL 25 (43A) 0.4 EMS	302	10	4	4	4	143	50	1	1	21.50	4	83.67	-8.09
DHL 53 POP 4 X EXP 124	300	10	5	4	4	164	69	2	1	22.50	5	90.57	-10.33
C316-7 X 1368-150GY (119)S	261	3	5	5	5	121	38	1	1	12.50	5	89.12	-11.35
1368 X C316-7	249	9	5	3	4	154	52	1	1	18.50	4	87.64	-8.71
TZIL 25 (40C) 0.4 EMS X TZIL 25 (60E) 0.4 EMS	248	9	4	5	3	164	56	1	1	24.00	4	82.69	-7.25
TZIL 25 (43A) 0.4 EMS X TZIL 25 (60E) 0.4 EMS	150	11	4	5	4	157	56	1	1	23.50	5	94.49	-11.31
LMI 102 X LMI 141	144	13	5	5	4	191	75	1	1	21.00	4	94.97	-11.10
1368-150GY (119)S X 1368	73	9	5	4	5	104	34	1	1	12.00	5	97.69	-12.95
TZIL 25 (60E) 0.4 EMS X 9006	39	9	5	7	5	151	57	1	1	20.50	5	97.59	-15.04
ABURO LEGON-CHK 1	2679	4	3	4	2	189	71	1	1	28.00	2	19.55	11.14
OBATANPA-CHK 2	605	9	4	4	4	205	84	2	1	25.50	5	68.56	-6.01
ABONTEM-CHK 3	1398	1	3	5	3	196	74	1	3	19.00	4	54.10	3.57
PIONEER-CHK 4	2564	3	3	2	2	180	76	2	3	20.50	4	19.80	10.46
GRAND MEAN	**1040**	**5**	**3**	**4**	**3**	**178**	**68**	**1**	**1**	**21.88**	**3**	**64.51**	
SE	**59.09**	**0.26**	**0.07**	**0.11**	**0.07**	**2.04**	**1.23**	**0.05**	**0.08**	**0.41**	**0.08**	**2.17**	

YLD, grain yield (kg/ha); ASI, anthesis-silking interval; ASI, anthesis-silking interval; LS, leaf senescence; PASP, plant aspect; PH, plant height (cm);EH, ear height (cm); SPAD, leaf chlorophyll content; EASP, ear aspect; SG, stay-green characteristic; 100KW, 100 kernel weight (g);RL, root lodging; SL, stalk lodging; YPN, yield penalty; BSI, base selection index; SE, standard error.

Table 4. 12: Top 15 and bottom 10 hybrids, including 4 checks, selected based on grain yield and other traits across optimum environments in 2023 and 2024.

HYBRID	YLD	ASI	SPAD	PASP	EH	PH	RL	SL	EASP	100KW
1368 X DHL 186 POP 2	4540	1	61	3	82	198	2	2	3	28
DHL 99 POP 2 X 9006	4380	1	54	3	78	191	2	2	3	28
LMI 135 X LMI 140	4272	2	48	3	90	198	3	4	3	28
DHL 46 POP 1 X LMI 135	4092	1	62	2	99	219	2	2	3	31
1368-150GY (119)S X DHL 184 POP 2	4091	1	51	3	63	177	2	2	4	33
HP020-4/1(A)/1/1 X DHL 46 POP 1	3972	1	59	2	81	200	2	2	3	28
LMI 102 X LMI 140	3924	2	52	3	88	204	2	3	3	31
DHL 99 POP 2 X EXP 124	3921	1	51	3	92	204	3	2	3	29
TZIL 25 (43A) 0.4 EMS X DHL 53 POP 4	3893	2	57	3	88	204	2	3	3	30
HP020-5/2(A)/1/7 X LMI 141	3892	2	55	3	91	198	2	2	3	27
LMI 140 X DHL 18 POP 1	3869	3	52	2	100	214	2	3	3	27
DHL 142 POP 1 X HP020-5/1(B)/1/7	3865	2	58	3	74	190	2	3	3	28
TZIL 25 (60E) 0.4 EMS X EXP 124	3804	2	55	3	79	192	2	2	3	33
TZIL 25 (43A) 0.4 EMS X DHL 99 POP 2	3789	1	63	2	91	210	3	3	3	29
HP020-5/1(B)/1/7 X LMI 141	3781	2	52	3	80	194	2	2	3	30
HP020-4/1(A)/1/1 X DHL 142 POP 1	2048	1	57	4	64	172	2	4	3	30
HP020-6/2(9)/9/6 X HP020-5/1(B)/1/7	2032	3	49	3	74	187	2	2	4	24
1368 X C316-7	2011	3	54	3	63	169	4	4	4	23
HP020-4/1(A)/1/1 X HP020-6/2(9)/9/6	1920	1	49	4	53	158	2	2	4	23
DHL 186 POP 2 X DHL 99 POP 2	1857	1	48	3	68	178	2	2	4	31
9006 X TZIL 25 (43A) 0.4 EMS	1852	3	49	4	60	162	2	2	4	25
DHL 53 POP 4 X 1368	1846	0	60	2	86	216	1	1	2	32
TZIL 25 (60E) 0.4 EMS X 9006	1639	2	44	4	64	161	2	2	4	27
EXP 124 X TZIL 25 (43A) 0.4 EMS	1472	0	67	4	66	175	1	1	3	27
TZIL 25 (40C) 0.4 EMS X TZIL 25 (60E) 0.4 EMS	1432	2	46	4	62	157	2	4	4	27
ABURO LEGON-CHK 1	3329	2	48	3	74	190	2	2	3	29
OBATANPA-CHK 2	1924	4	43	3	85	199	3	2	4	32
ABONTEM-CHK 3	3046	2	51	3	79	192	2	2	3	29
PIONEER-CHK 4	3197	2	63	3	75	176	2	2	4	31
GRAND MEAN	**2979**	**2**	**53**	**3**	77	**189**	**2**	**2**	**3**	**28**
S.E	**50.49**	**0.06**	**0.43**	**0.04**	**0.79**	**1.32**	**0.06**	**0.06**	**0.04**	**0.18**

YLD, grain yield (kg/ha); ASI, anthesis-silking interval; ASI, anthesis-silking interval; PASP, plant aspect; PH, plant height (cm);EH, ear height (cm); SPAD, leaf chlorophyll content; EASP, ear aspect; RL, root lodging; SL, stalk lodging; 100KW, 100 kernel weight (g); SE, standard error.

Table 4. 13: Top 15 and bottom 10 hybrids, including 4 checks, selected based on grain yield and other traits across all environments in 2023 and 2024.

HYBRID	YLD	DYANTH	DYSK	ASI	SPAD	PASP	EH	PH	EASP	100KW
DHL 53 POP 4 X 1368	3357	54	54	0	65	2	86	216	2	32
LMI 135 X HP020-4/1(A)/1/1	3065	54	54	0	59	3	90	213	4	25
LMI 102 X LMI 140	2918	54	56	2	44	3	88	207	3	29
LMI 141 X DHL 142 POP 1	2819	51	53	2	65	2	107	251	3	30
DHL 53 POP 4 X 9006	2797	53	54	1	61	3	82	224	3	33
DHL 99 POP 2 X 9006	2794	56	57	1	43	3	70	177	3	26
HP020-6/2(9)/9/6 X LMI 102	2738	56	57	1	69	3	78	232	3	29
LMI 135 X LMI 140	2714	54	57	3	40	3	82	193	3	26
HP020-4/1(A)/1/1 X DHL 46 POP 1	2616	57	61	3	44	3	78	190	3	25
DHL 99 POP 2 X EXP 124	2589	54	56	2	40	3	83	188	3	27
1368 X DHL 186 POP 2	2553	54	57	3	46	3	79	189	3	27
LMI 140 X DHL 18 POP 1	2499	50	53	3	42	3	91	200	3	25
DHL 186 POP 2 X TZIL 25 (60E) 0.4 EMS	2483	53	55	2	47	3	87	195	3	25
1368-150GY (119)S X DHL 184 POP 2	2475	55	59	4	39	4	64	179	4	27
TZIL 25 (43A) 0.4 EMS X DHL 53 POP 4	2368	55	58	3	43	3	84	197	3	29
TZIL 25 (43A) 0.4 EMS X TZIL 25 (60E) 0.4 EMS	1404	55	60	5	40	3	69	176	4	26
9006 X TZIL 25 (43A) 0.4 EMS	1398	58	62	4	42	4	58	157	4	26
C316-7 X 1368-150GY (119)S	1358	60	63	3	41	4	61	163	4	22
HP020-6/2(9)/9/6 X HP020-5/1(B)/1/7	1227	58	62	5	39	4	71	182	4	25
DHL 53 POP 4 X DHL 184 POP 2	1209	57	62	5	36	4	72	176	4	24
9006 X TZIL 25 (40C) 0.4 EMS	1201	59	62	3	39	4	55	151	4	22
HP020-4/1(A)/1/1 X HP020-6/2(9)/9/6	1160	61	63	2	41	4	56	159	4	20
1368 X C316-7	1123	56	61	5	43	4	60	169	4	19
TZIL 25 (60E) 0.4 EMS X 9006	1014	57	61	4	40	4	64	161	4	24
TZIL 25 (40C) 0.4 EMS X TZIL 25 (60E) 0.4 EMS	838	57	61	5	39	4	58	152	4	26
ABURO LEGON-CHK 1	2531	54	57	3	40	3	73	185	3	26
OBATANPA-CHK 2	1304	56	61	5	36	4	86	195	4	29
ABONTEM-CHK 3	2064	51	52	2	42	3	74	189	4	25
PIONEER-CHK 4	2366	55	57	2	48	3	79	181	4	27
GRAND MEAN	**1986**	**55**	**58**	**3**	**43**	**3**	**74**	**183**	**3**	**26**
S.E	**38.75**	**0.12**	**0.15**	**0.07**	**0.39**	**0.03**	**0.53**	**1.06**	**0.03**	**0.17**

YLD, grain yield (kg/ha); ASI, anthesis-silking interval; ASI, anthesis-silking interval; PASP, plant aspect; PH, plant height (cm);EH, ear height (cm); SPAD, leaf chlorophyll content; EASP, ear aspect; 100KW, 100 kernel weight (g); SE, standard error.

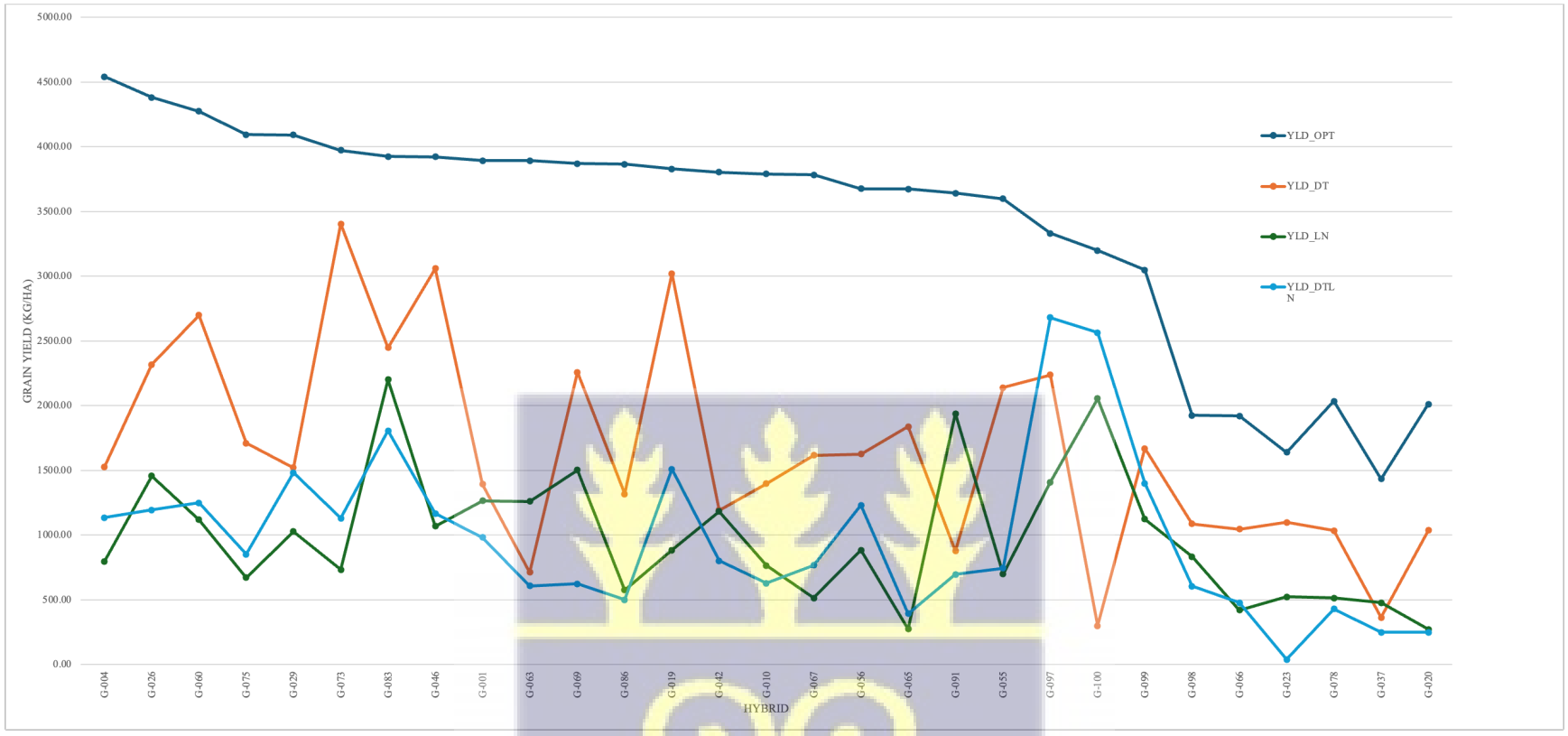

Figure 4. 1: Performance of 29 selected hybrids, including 4 checks, under optimal, drought, low N and combined drought and low N environments in 2023 and 2024.

CODE	HYBRID	CODE	HYBRID	CODE	HYBRID
G-004	1368 X DHL 186 POP 2	G-069	LMI 140 X DHL 18 POP 1	G-097	ABURO LEGON - CHK 1
G-026	DHL 99 POP 2 X 9006	G-086	DHL 142 POP 1 X HP020-5/1(B)/1/7	G-100	PIONEER-CHK 4
G-060	LMI 135 X LMI 140	G-019	TZIL 25 (40C) 0.4 EMS X DHL 99 POP 2	G-099	ABONTEM-CHK 3
G-075	DHL 46 POP 1 X LMI 135	G-042	TZIL 25 (60E) 0.4 EMS X EXP 124	G-098	OBATANPA - CHK 2
G-029	1368-150GY (119)S X DHL 184 POP 2	G-010	TZIL 25 (43A) 0.4 EMS X DHL 99 POP 2	G-066	HP020-4/1(A)/1/1 X HP020-6/2(9)/9/6
G-073	HP020-4/1(A)/1/1 X DHL 46 POP 1	G-067	HP020-5/1(B)/1/7 X LMI 141	G-023	TZIL 25 (60E) 0.4 EMS X 9006
G-083	LMI 102 X LMI 140	G-056	LMI 135 X LMI 141	G-078	HP020-6/2(9)/9/6 X HP020-5/1(B)/1/7
G-046	DHL 99 POP 2 X EXP 124	G-065	DHL 18 POP 1 X HP020-5/1(B)/1/7	G-037	TZIL 25 (40C) 0.4 EMS X TZIL 25 (60E) 0.4 EMS
G-001	TZIL 25 (43A) 0.4 EMS X DHL 53 POP 4	G-091	LMI 102 X HP020-4/1(A)/1/1	G-020	1368 X C316-7
G-063	HP020-5/2(A)/1/7 X LMI 141	G-055	HP020-5/2(A)/1/7 X LMI 144		

4.4.3 Genotype-by-environment interaction (G x E) and stability of the hybrids

Using the mean grain yield of 29 selected hybrids (top 15, bottom 10 and four checks including checks), GGE biplots were generated across the seven environments. The genotype main effects and the genotype x environment interaction (GGE) biplot explained 75.54% of the total variation in grain yield across the environments with the two axes, PC1 and PC2, constituting 60.83% and 14.71% respectively, of the captured variation. The *'which won where'* polygon view grouped the hybrids into mega-environments with each having winning hybrid(s) at the vertices of the polygon (Figure 4.2).The perpendicular lines to the polygon sides divide the biplot into sectors, which suggests mega-environments where different genotypes won.

Three mega-environments were revealed based on environment groupings where E2 and E6, formed the first group where G20, LMI 102 X LMI 140 , was the winning hybrid. The second mega-environment had E1 and E3 where G2 (1368 X DHL 186 POP 2) and G15 (HP020-4/1(A)/1/1 X DHL 46 POP 1) were the superior hybrids. E4, E5 and E7 were grouped together as the third mega-environment associated with G25 (TZIL 25 (40C) 0.4 EMS X TZIL 25 (60E) 0.4 EMS) being the superior hybrid in that environment. Among the test environments, E2 and E6 were the most discriminating, whereas E4, E5 and E7 were closely associated, indicating similarity in their genotype response (Figure 4.4).

The *'mean vs stability'* GGE biplot was used to identify high yielding and stable hybrids across all environments (Figure 4.3). The average environment coordination (AEC) abscissa represents the mean performance of each hybrid, while the ordinate (error bars) indicates stability. The longer the error bar, the greater the instability. Hybrids positioned to the right of the AEC exhibited above-average mean yield, while those to the left recorded below-average perfromance.

G20 (LMI 102 X LMI 140) was identified as the highest yielding hybrid though less stable. G3 (1368-150GY (119)S X DHL 184 POP 2), G11 (DHL 99 POP 2 X 9006) and G12 (DHL 99 POP 2 X EXP 124) were the most stable hybrids identified.

Among all the test environments, E5 (Kwadaso LN, major rainy season 2024) was the least discriminating of the hybrids as it had the shortest vector from the biplot origin as observed in the '*discriminating vs representativeness*' biplot (Figure 4.4). E2 (Legon OPT, major rainy season 2024), E3 (Kwadaso OPT, major rainy season 2024) and E6 (Legon LN, major rainy season 2024) had the highest discriminating ability of the hybrids. The environments , E6 and E3 , are the most representative of their respective mega-environments. These environments, E6 and E3, as well as E5 and E2, can be used as testing locations.

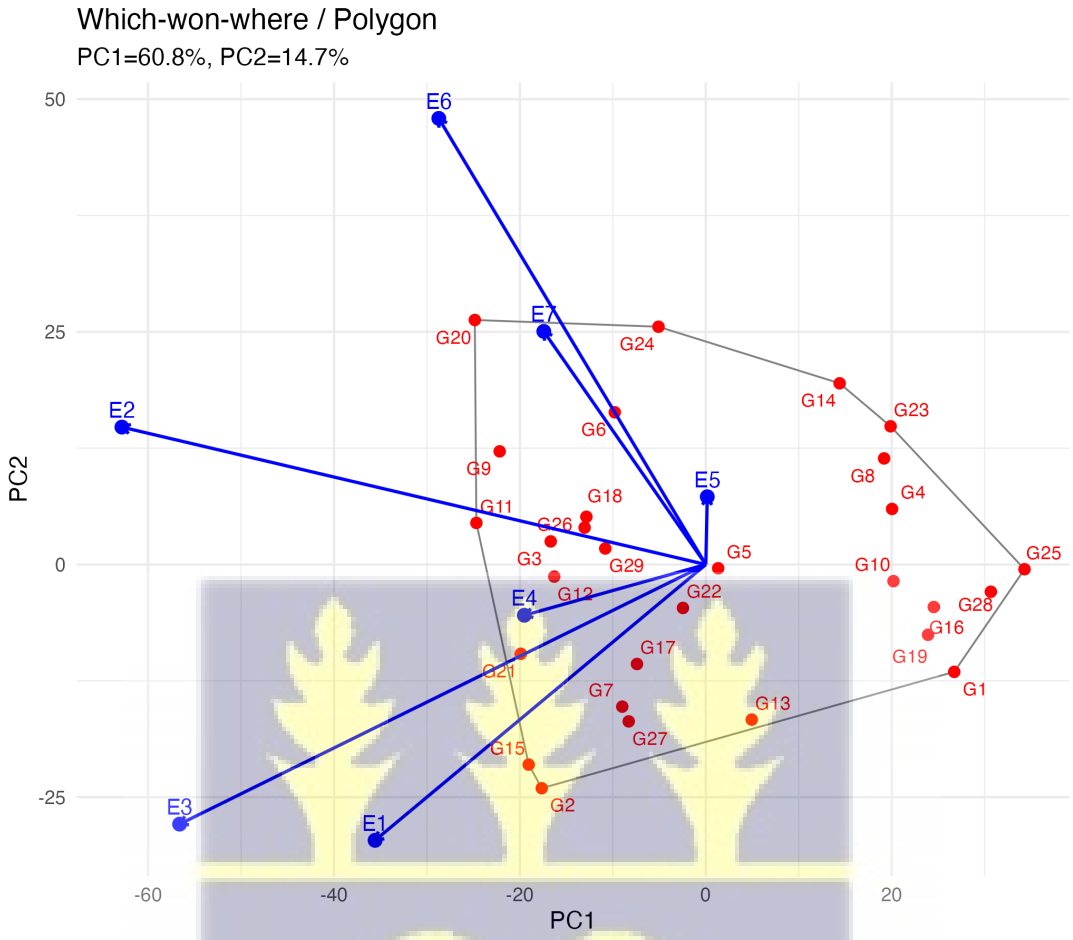

Figure 4. 2: A *'which won where/what'* GGE biplot of 29 selected hybrids, including checks, across DT, LN, DTLN and optimal environments in 2023 and 2024.

Code	Environment	Code	Hybrid	Code	Hybrid
E1	Legon OPT, minor season 2023	G1	1368 X C316-7	G16	HP020-4/1(A)/1/1 X HP020-6/2(9)/9/6
E2	Legon OPT, major season 2024	G2	1368 X DHL 186 POP 2	G17	HP020-5/1(B)/1/7 X LMI 141
E3	Kwadaso OPT, major season 2024	G3	1368-150GY (119)S X DHL 184 POP 2	G18	HP020-5/2(A)/1/7 X LMI 141
E4	Legon DT, dry season 2023	G4	9006 X TZIL 25 (43A) 0.4 EMS	G19	HP020-6/2(9)/9/6 X HP020-5/1(B)/1/7
E5	Kwadaso LN, major season 2024	G5	ABONTEM-CHK 3	G20	LMI 102 X LMI 140
E6	Legon LN, major season 2024	G6	ABURO LEGON-CHK 1	G21	LMI 135 X LMI 140
E7	Legon DTLN, dry season 2023	G7	DHL 142 POP 1 X HP020-5/1(B)/1/7	G22	LMI 140 X DHL 142 POP 1
		G8	DHL 186 POP 2 X DHL 99 POP 2	G23	OBATANPA-CHK 2
		G9	DHL 46 POP 1 X LMI 135	G24	PIONEER-CHK 4
		G10	DHL 53 POP 4 X DHL 184 POP 2	G25	TZIL 25 (40C) 0.4 EMS X TZIL 25 (60E) 0.4 EMS
		G11	DHL 99 POP 2 X 9006	G26	TZIL 25 (43A) 0.4 EMS X DHL 53 POP 4
		G12	DHL 99 POP 2 X EXP 124	G27	TZIL 25 (43A) 0.4 EMS X DHL 99 POP 2
		G13	EXP 124 X TZIL 25 (40C) 0.4 EMS	G28	TZIL 25 (60E) 0.4 EMS X 9006
		G14	HP020-4/1(A)/1/1 X DHL 142 POP 1	G29	TZIL 25 (60E) 0.4 EMS X EXP 124
		G15	HP020-4/1(A)/1/1 X DHL 46 POP 1		

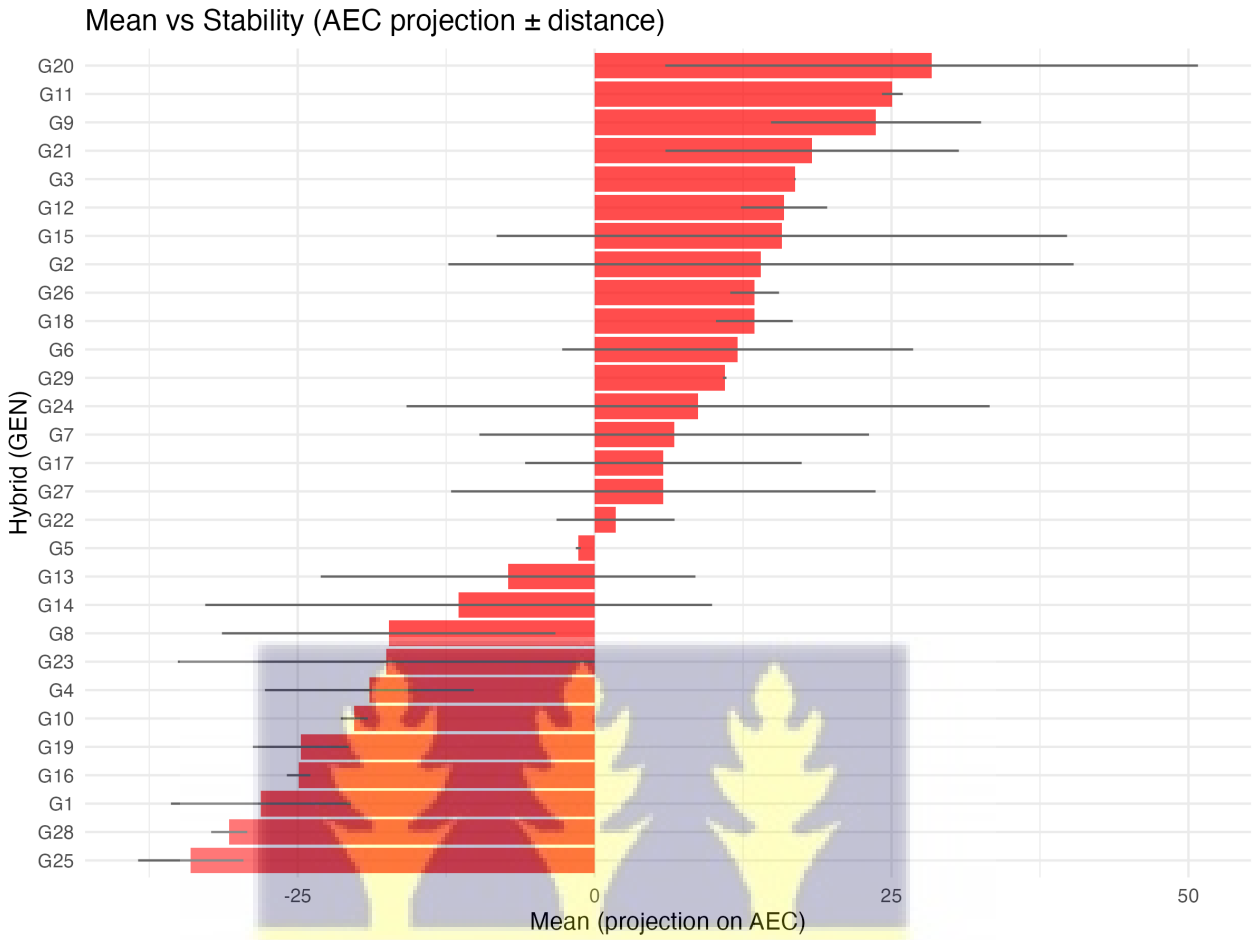

Figure 4. 3: An entry/tester focused GGE biplot of twenty-nine selected hybrids, including checks, across DT, LN, DTLN and optimal environments in 2023 and 2024.

Code	Environment	Code	Hybrid	Code	Hybrid
E1	Legon OPT, minor season 2023	G1	1368 X C316-7	G16	HP020-4/1(A)/1/1 X HP020-6/2(9)/9/6
E2	Legon OPT, major season 2024	G2	1368 X DHL 186 POP 2	G17	HP020-5/1(B)/1/7 X LMI 141
E3	Kwadaso OPT, major season 2024	G3	1368-150GY (119)S X DHL 184 POP 2	G18	HP020-5/2(A)/1/7 X LMI 141
E4	Legon DT, dry season 2023	G4	9006 X TZIL 25 (43A) 0.4 EMS	G19	HP020-6/2(9)/9/6 X HP020-5/1(B)/1/7
E5	Kwadaso LN, major season 2024	G5	ABONTEM-CHK 3	G20	LMI 102 X LMI 140
E6	Legon LN, major season 2024	G6	ABURO LEGON-CHK 1	G21	LMI 135 X LMI 140
E7	Legon DTLN, dry season 2023	G7	DHL 142 POP 1 X HP020-5/1(B)/1/7	G22	LMI 140 X DHL 142 POP 1
		G8	DHL 186 POP 2 X DHL 99 POP 2	G23	OBATANPA-CHK 2
		G9	DHL 46 POP 1 X LMI 135	G24	PIONEER-CHK 4
		G10	DHL 53 POP 4 X DHL 184 POP 2	G25	TZIL 25 (40C) 0.4 EMS X TZIL 25 (60E) 0.4 EMS
		G11	DHL 99 POP 2 X 9006	G26	TZIL 25 (43A) 0.4 EMS X DHL 53 POP 4
		G12	DHL 99 POP 2 X EXP 124	G27	TZIL 25 (43A) 0.4 EMS X DHL 99 POP 2
		G13	EXP 124 X TZIL 25 (40C) 0.4 EMS	G28	TZIL 25 (60E) 0.4 EMS X 9006
		G14	HP020-4/1(A)/1/1 X DHL 142 POP 1	G29	TZIL 25 (60E) 0.4 EMS X EXP 124
		G15	HP020-4/1(A)/1/1 X DHL 46 POP 1		

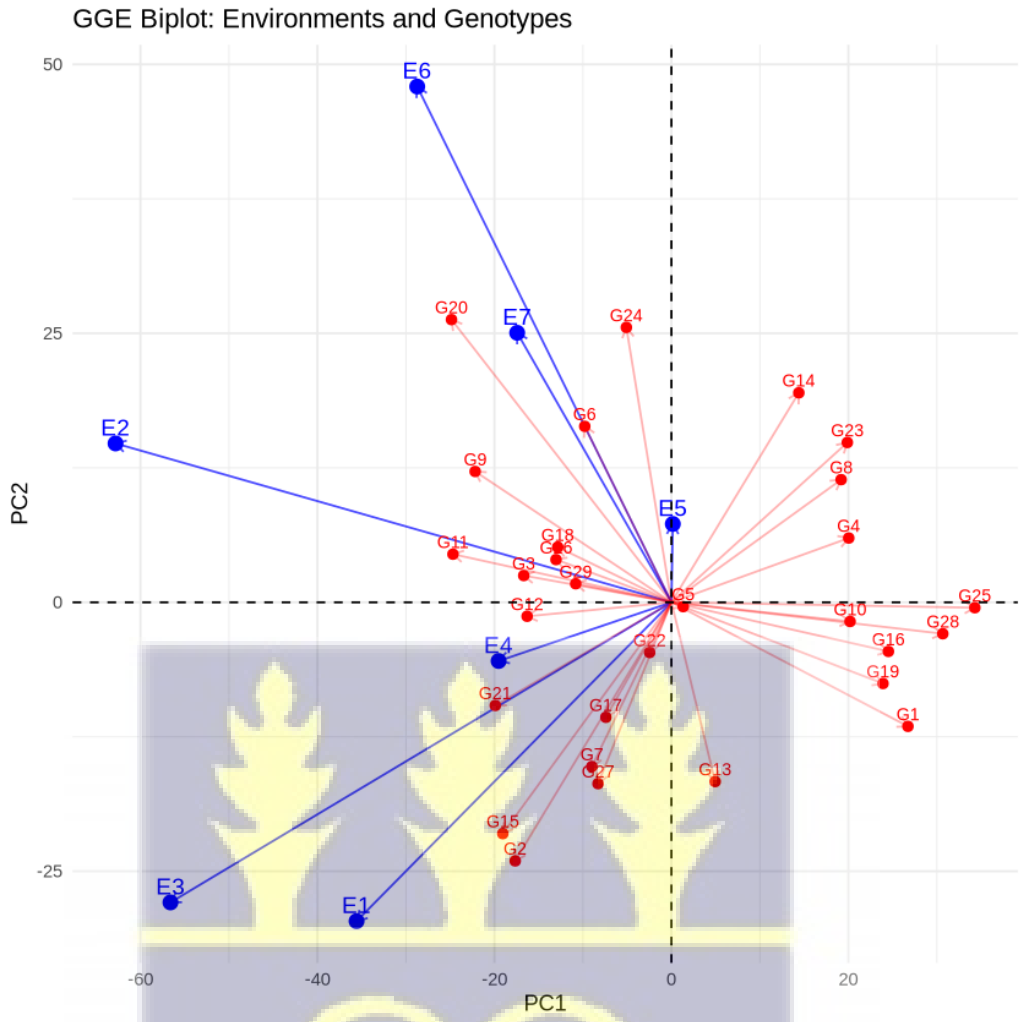

Figure 4. 4: Discriminating vs. representativeness view of GGE biplot of twenty-nine selected hybrids, including checks, across DT, LN, DTLN and optimal environments in 2023 and 2024.

Code	Environment	Code	Hybrid	Code	Hybrid
E1	Legon OPT, minor season 2023	G1	1368 X C316-7	G16	HP020-4/1(A)/1/1 X HP020-6/2(9)/9/6
E2	Legon OPT, major season 2024	G2	1368 X DHL 186 POP 2	G17	HP020-5/1(B)/1/7 X LMI 141
E3	Kwadaso OPT, major season 2024	G3	1368-150GY (119)S X DHL 184 POP 2	G18	HP020-5/2(A)/1/7 X LMI 141
E4	Legon DT, dry season 2023	G4	9006 X TZIL 25 (43A) 0.4 EMS	G19	HP020-6/2(9)/9/6 X HP020-5/1(B)/1/7
E5	Kwadaso LN, major season 2024	G5	ABONTEM-CHK 3	G20	LMI 102 X LMI 140
E6	Legon LN, major season 2024	G6	ABURO LEGON-CHK 1	G21	LMI 135 X LMI 140
E7	Legon DTLN, dry season 2023	G7	DHL 142 POP 1 X HP020-5/1(B)/1/7	G22	LMI 140 X DHL 142 POP 1
		G8	DHL 186 POP 2 X DHL 99 POP 2	G23	OBATANPA-CHK 2
		G9	DHL 46 POP 1 X LMI 135	G24	PIONEER-CHK 4
		G10	DHL 53 POP 4 X DHL 184 POP 2	G25	TZIL 25 (40C) 0.4 EMS X TZIL 25 (60E) 0.4 EMS
		G11	DHL 99 POP 2 X 9006	G26	TZIL 25 (43A) 0.4 EMS X DHL 53 POP 4
		G12	DHL 99 POP 2 X EXP 124	G27	TZIL 25 (43A) 0.4 EMS X DHL 99 POP 2
		G13	EXP 124 X TZIL 25 (40C) 0.4 EMS	G28	TZIL 25 (60E) 0.4 EMS X 9006
		G14	HP020-4/1(A)/1/1 X DHL 142 POP 1	G29	TZIL 25 (60E) 0.4 EMS X EXP 124
		G15	HP020-4/1(A)/1/1 X DHL 46 POP 1		

4.4.4 Interrelationship between traits across test environments and among test environments for grain yield

Under drought environment, significant positive correlation was observed between yield (YLD) and leaf chlorophyll content (SPAD), plant height (PH), ear height (EH) and 100 kernel weight (X100KW) (Table 4.14). Significant negative correlations were observed between YLD and plant aspect (PASP), ear aspect (EASP) and leaf senescence (LS). Significant positive correlation was observed between PH and EH under drought environment.

Significant negative correlation was observed between YLD and all traits except for SPAD, PH and EH under low N environment (Table 4.15). Significant positive correlation was also observed between LS and stay-green characteristic (STGR). However, significant negative correlation was observed between SPAD and LS as well as STGR.

With the exception of SPAD, PH, EH, 100KW and EPP, yield was negatively correlated with all traits under combined drought and low N environment (Table 4.16). Significant negative correlation was observed between SPAD and STGR as well as LS. Significant positive correlation was observed between STGR and DYANTH, DYSK, ASI and PASP.

The correlation coefficients based on grain yield among all test environments indicated a significant positive correlation between E1 (Legon OPT, minor rainy season 2023) and E2 (Legon OPT, major season 2024), E2 and E3 (Kwadaso OPT, major season 2024), E3 and E4 (Legon DT, dry season 2023) and E6 (Legon LN, major season 2024) and E7 (Legon DTLN, dry season 2023) as observed in Table 4.17. A negative correlation was observed between E1 and E5 (Kwadaso LN, major rainy season 2024).

Table 4. 14: Estimates of phenotypic correlation coefficients of grain yield and other agronomic traits evaluated under drought environment in Legon, 2023.

Traits	DYANTH	DYSK	ASI	PASP	LS	SPAD	PH	EH	100KW	EPP	EASP	YLD
DYSK	0.83**											
ASI	0.15	0.67**										
PASP	0.39**	0.57**	0.50**									
LS	0.06	0.31**	0.48**	0.60**								
SPAD	0.12	-0.03	-0.21*	-0.22*	-0.26							
PH	-0.47**	-0.52**	-0.30**	-0.58**	-0.30**	0.00						
EH	-0.38**	-0.36**	-0.13	-0.43**	-0.19	-0.07	0.76**					
100KW	0.02	-0.07	-0.15	-0.45**	-0.50**	0.25*	0.19	0.06				
EPP	0.15	0.23*	0.21*	0.14	0.12	0.07	0.01	0.06	0.12			
EASP	0.21*	0.44**	0.50**	0.63**	0.48**	-0.30**	-0.35**	-0.27**	-0.38**	-0.12		
YLD	-0.39**	-0.61**	-0.57**	-0.73**	-0.65**	0.30**	0.54**	0.38**	0.45**	-0.13	-0.74**	

*DYANTH, days to anthesis; DYSK, days to silking; ASI, anthesis-silking interval; PASP, plant aspect; LS, leaf senescence; SPAD, leaf chlorophyll content PH, plant height (cm);EH, ear height (cm); 100KW, 100 kernel weight (g); EPP, ears per plant; EASP, ear aspect; YLD, grain yield (kg/ha); *=significant at 0.05 probability level, **=highly significant at 0.01 level*

Table 4. 15: Estimates of phenotypic correlation coefficients of grain yield and other agronomic traits evaluated under low N environments in Legon and Kwadaso, 2024.

Traits	DYANTH	DYSK	ASI	PASP	STGR	LS	SPAD	PH	EH	EASP	YLD
DYSK	0.91**										
ASI	0.21**	0.60**									
PASP	0.71**	0.72**	0.33**								
STGR	0.60**	0.68**	0.43**	0.77**							
LS	0.43**	0.43**	0.19**	0.45**	0.59**						
SPAD	-0.48**	-0.53**	-0.32**	-0.59**	-0.65**	-0.57**					
PH	-0.77**	-0.72**	-0.21**	-0.79**	-0.66**	-0.44**	0.56**				
EH	-0.74**	-0.70**	-0.22**	-0.76**	-0.60**	-0.21**	0.41**	0.88**			
EASP	0.59**	0.67**	0.44**	0.67**	0.66**	0.39**	-0.53**	-0.63**	-0.61**		
YLD	-0.66**	-0.75**	-0.48**	-0.75**	-0.71**	-0.44**	0.57**	0.69**	0.64**	-0.79**	

*DYANTH, days to anthesis; DYSK, days to silking; ASI, anthesis-silking interval; PASP, plant aspect; STGR, stay-green characteristic; LS, leaf senescence; SPAD, leaf chlorophyll content PH, plant height (cm);EH, ear height (cm); EPP, ears per plant; EASP, ear aspect; YLD, grain yield (kg/ha); *=significant at 0.05 probability level, **=highly significant at 0.01 level*

Table 4. 16: Estimates of phenotypic correlation coefficients of grain yield and other agronomic traits evaluated under combined drought and low N environments in Legon, 2023.

TRAIT	DYANTH	DYSK	ASI	PASP	STGR	LS	SPAD	PH	EH	100KW	EPP	EASP	YLD
DYSK	0.82**												
ASI	0.16	0.69**											
PASP	0.44**	0.61**	0.49**										
STGR	0.26**	0.46**	0.47**	0.83**									
LS	-0.02	0.07	0.14	0.32**	0.53**								
SPAD	-0.01	-0.12	-0.19	-0.36**	-0.53**	-0.47**							
PH	-0.46**	-0.47**	-0.23*	-0.69**	-0.50**	0.00	0.10						
EH	-0.50**	-0.51**	-0.25*	-0.62**	-0.36**	0.11	-0.07	0.88**					
100KW	0.15	0.13	0.05	-0.23*	-0.36**	-0.30**	0.33**	0.21*	-0.02				
EPP	-0.04	-0.08	-0.09	-0.07	-0.07	0.09	-0.11	0.16	0.09	0.11			
EASP	0.15	0.32**	0.37**	0.49**	0.54**	0.38**	-0.41**	-0.33**	-0.23**	-0.30**	-0.22*		
YLD	-0.36	-0.61	-0.59	-0.72	-0.72	-0.39	0.47	0.42	0.37	0.26	0.04	-0.65*	

*DYANTH, days to anthesis; DYSK, days to silking; ASI, anthesis-silking interval; PASP, plant aspect; STGR, stay-green characteristic; LS, leaf senescence; SPAD, leaf chlorophyll content PH, plant height (cm);EH, ear height (cm): 100KW, 100 kernel weight (g); EPP, ears per plant; EASP, ear aspect; YLD, grain yield (kg/ha); *=significant at 0.05 probability level, **=highly significant at 0.01 level*

Table 4. 17: Estimates of phenotypic correlation coefficients of the varying environments based on grain yield.

Environment	E1	E2	E3	E4	E5	E6	E7
E1							
E2	0.25*						
E3	0.17	0.32**					
E4	0.08	0.11	0.33**				
E5	-0.19	0.05	0.01	0.11			
E6	0.05	0.16	0.12	0.11	0.16		
E7	0.01	0.18	0.16	0.25	0.42	0.40**	

E1, Legon OPT, minor season 2023; E2, Legon OPT, major season 2024; E3, Kwadaso OPT, major season 2024; E4, Legon DT, dry season 2023; E5, Kwadaso LN, major season 2024; E6, Legon LN, major season 2024; E7, Legon DTLN, dry season 2023.

4.4 Discussion

The highly significant differences observed among sources of variation for most measured traits across the various environments confirm the presence of substantial genetic variability among the inbred lines and their hybrids. This variability provides opportunities for effective selection and maize improvement under contrasting environments (Alam *et al.*, 2022; Dube *et al.*, 2024; Tefera *et al.*, 2020; L. Yadesa *et al.*, 2024). The significant replication and block effects observed across environments indicate environmental heterogeneity within experimental sites, underscoring the importance of the alpha-lattice design in improving precision and minimizing experimental error (Akinwale *et al.*, 2021; Alivelu *et al.*, 2020).

The significant GCA_f, GCA_m and SCA effects for grain yield and other traits in the contrasting environments indicated variations in the performance of the inbred lines used as parents in the hybrid development. This also suggests the importance additive and non-additive gene actions for the inheritance of grain yield and other traits under drought, low N, combined drought and low N, and optimum environments. Similar findings were reported by Badu-Apraku *et al.* (2016), Bhadmus *et al.* (2021), Owusu *et al.* (2023), under single stress, combined stress and optimum environments. However, the non-significant SCA effects observed for yield and flowering traits under combined drought and low nitrogen condition imply that these characters were largely governed by additive rather than dominance effects. Hence, breeding programmes could prioritize additive gene effects when selecting for these traits under drought and low N environments. This is crucial as additive gene effects are typically more predictable and can be effectively utilized through traditional selection methods, resulting in more stable and consistent improvements in crop productivity under stress environments. Badu-Apraku, Fakorede, Talabi, *et al.* (2016b) highlighted that while non-additive effects influence grain yield, flowering traits in certain tropical

maize populations exposed to drought stress were predominantly governed by additive gene action. This is corraborated by Elmyhun *et al.* (2024) who affirmed the prevalence of additive gene action for several traits under managed drought stress conditions, indicating that these effects can be effectively utilized in breeding programmes for enhanced maize varieties.

The significant interactions of GCA-female, GCA-male and SCA with the environment for most traits across environments further confirms the differential response of the hybrids to environmental conditions, reflecting the complex influence of environmental stress on trait expression. Badu-Apraku *et al.* (2020a) conducted genetic studies on provitamin-A and found that GCA interactions with the environment significantly affect trait expression, indicating that both additive and non-additive genetic controls are important in the inheritance of these traits across different conditions. Their findings highlight that GCA and SCA can influence hybrid responsiveness to environmental changes, which is crucual for predicting performance based on GCA. Akaogu *et al.* (2017a) noted significant GCA x environment interactions, which are critical for understanding yield responses to different environmental conditons, primarily in drought and rain-fed scenarios. They reported that significant SCA x environment interactions imply that hybrid performance can be inconsistent as influenced by environmental conditions, indicating the complexity of these interactions governing trait expression.

The moderate to high broad-sense heritability values recorded for key agronomic traits such as plant height, days to anthesis and grain yield show that these traits are genetically controlled and could respond favourably to selection (Naggar *et al.*, 2020; Okunlola *et al.*, 2023b). In contrast, the generally low narrow-sense heritability estimates across environments indicate that additive genetic variance accounted for only a small proportion of the total phenotypic variance. This pattern is expected in hybrid populations derived from diverse parental lines, where dominance

and epistatic effects, as well as G x E interactions, contribute strongly to observed variation (Kristensen *et al.*, 2023; Monir & Zhu, 2018; Shirinpour *et al.*, 2023; Tsouris *et al.*, 2024).

Under combined drought and low N, the significant differences among environment and parental lines highlights the strong influence of stress intensity and resource limitation on trait expression. M. Abid *et al.* (2016) investigated the effects of drought stress under various nitrogen levels in wheat. Findings from their study emphasized that drought conditions during the vegetative phase adversely affected grain yield and spike fertility, with reduced grain per spike and grain weight under limited N conditions. Similarly, J. Yan *et al.* (2023) observed that ethylene regulates tolerance to combined drought and low nitrogen in wheat. They found that the combined stress from drought and nitrogen deficiency led to an increase in proteins related to energy, carbohydrate and amino acid metabolism, suggesting a complex network of responses that influence traits vital for growth and development under stress. In maize, drought and nitrogen deficiency often exacerbate yield reduction through delayed anthesis, increased ASI and accelerated leaf senescence, thereby reducing assimilate partitioning to the developing ear (Akaogu *et al.*, 2017b; Bharathi *et al.*, 2021; Shen *et al.*, 2025). The predominance of additive genetic control for grain yield and flowering traits under combined drought and low N suggests that recurrent selection for additive effects could be effective for improving tolerance to combined stress while the significant SCA effects for secondary traits such as plant height, 100-seed kernel weight and stay-green characteristic imply that specific parental combinations can still be exploited to capture heterotic responses to these traits.

The significant environmental main effects across all test locations confirm the variability of the environments emphasizing the need to test in multiple environments to identify stable and high-yielding hybrids across locations. The significant G x E inteactions observed for yield and

associated traits further emphasize the value of multi-environment evaluation to identify broadly adapted hybrids (Boreddy *et al.*, 2020b; Pour-Aboughadareh *et al.*, 2022). The absence of ENV x FEMALE x MALE interactions implies that although hybrid performance varied, the rank order of specific combinations was stable, simplifying the selection of superior hybrids for both optimal and stress conditions (Adham *et al.*, 2022; Kunwar *et al.*, 2024; Mebratu *et al.*, 2019).

Assessment of the 96 yellow and white single cross hybrids and 4 checks under drought, low N, combined drought and low N and optimum environments was necessary to identify best performing hybrids under each and across environments. Under drought, a wide range of grain yield reduction of 2 to 93%, and an average of 45% was observed. Other studies have reported yield reductions ranging from 24% to 90%, depending on the timing and severity of the drought stress imposed during flowering (Ahmad *et al.*, 2020; Ertiro *et al.*, 2017; Mamadou Mory *et al.*, 2014). These results demonstrated that the drought imposed at flowering was sufficiently severe to effectively discriminate between drought-tolerant and susceptible hybrids thereby enabling the identification and selection of superior hybrids. The average grain yield of the top ten single-cross hybrids was higher than the best check, Aburo Legon, under drought indicating that the developed single-cross hybrids were superior for drought stress tolerance and had greater stability than the commercial checks. Daryanto *et al.* (2016b) highlighted that modern maize hybrids have been bred to withstand drought conditions better than older varieties thereby leading to higher yields even under stress. This agrees with results from Menkir *et al.* (2024b) who reported that significant genetic gains have been made in developing drought-tolerant hybrids, which consistently yield higher that older varieties in various environments.

The use of the Base Selection Index (BSI) in this study proved to be a valuable tool for identifying genotypes under stress environments. Unlike single-trait selection that relies solely on grain yield,

the BSI integrates key agronomic and adaptive traits, such as yield components, number of ears per plant, ASI, plant and ear aspects, leaf senescence and stay-green characteristics, into a single quantitative value representing overall plant performance. This multi-trait approach allows for a more comprehensive evaluation of genotypes, balancing yield potential with stress-adaptive efficiency. Research by Talabi *et al.* (2017) supports this perspective, having identified key traits such as ear aspect, ears per plant, plant aspect and ASI as critical factors contributing to yield variation under drought stress. Their findings advocate for the inclusion of these traits into breeding indices, as a multi-trait selection strategy can significantly enhance the resilience of maize genotypes in adverse environments. Furthermore, Oyekunle *et al.* (2015) emphasized the necessity of evaluating hybrids under varied conditions, revealing that using an index incorporating multiple traits leads to better insights into how genotypes perform under environmental stresses.

Under stress conditions, performance trade-offs are common, where genotypes that yield highly under optimal condition may not maintain performance under stress because of unfavurable secondary traits such as prolonged ASI or rapid leaf senescence. The BSI addresses this limitation by assigning positive weights to yield-enhancing and tolerance traits, while penalizing undesirable ones, thereby ensuring that selection captures both productivity and resilience (Ramaswamy *et al.*, 2024; Rezende *et al.*, 2020).

Using the base index for selection under drought, HP020-4/1(A) X DHL 46 POP 1 was identified as the superior hybrid out-yielding the best check, Aburo Legon, by 41%. The ten most promising hybrids identified under drought using the base index were HP020-4/1(A) X DHL 46 POP 1, HP020-4/1(A)/1/1 X DHL 18 POP 1, HP020-6/2(9)/9/6 X LMI 135, TZIL 25 (40C) 0.4 EMS X DHL 99 POP 2, EXP 124 X DHL 186 POP 2, DHL 142 POP 1 X LMI 135, DHL 46 POP 1 X

HP020-5/2(A)/1/7, HP020-5/1(B)/1/7 X LMI 140, DHL 184 POP 2 X TZIL 25 (43A) 0.4 EMS and LMI 135 X LMI 140. Eight out of the ten superior hybrids under drought had at least one drought-tolerant parent while six out of the worst performing lines had both parents being susceptible to drought. These results are supported by other studies such as Obeng-Bio *et al.* (2020) who highlighted that the best yielding provitamin A quality protein maize hybrids were derived from inbred lines that exhibited high nutritional quality and drought tolerance. Similarly, Iseghohi *et al.* (2022) found that many hybrids derived from recurrent selection of drought-tolerant parental lines demonstrated desirable grain yields under drought stress, suggesting that the genetic background of the parental lines plays a crucial role in hybrid performance. Amegbor *et al.* (2020) also noted that hybrids derived from inbred lines lacking drought tolerant genes demonstrated reduced grain yield under drought conditions, emphasizing the detrimental impact of using susceptible parental lines.

Across low N environments, a range of grain yield reduction of 21 to 94%, and an average of 64% was observed. This is corroborated by Bhadmus *et al.* (2021) who conducted a genetic analysis of maize hybrids under low nitrogen conditions and found that grain yield reductions were substantial, with some hybrids experiencing reductions as high as 94% compared to optimal nitrogen conditions. Similarly, Madagoudra *et al.* (2021) reported reductions in grain yield ranging from 43% to 74% under low nitrogen conditions, highlighting the significant impact of nitrogen deficiency on maize productivity. The average grain yield of the top ten single-cross hybrids was higher than three of the checks, Aburo Legon, Abontem and Obatanpa but not Pioneer which was not significantly different from the top performing hybrid, LMI 102 X LMI 140. LMI 102 X LMI 140, the highest yielding hybrid, out-performed Pioneer by 7%. The top ten best hybrids selected across low N environment using the base index were LMI 102 X HP020-4/1(A)/1/1, LMI 102 X

LMI 140, DHL 99 POP 2 X DHL 184 POP 2, DHL 186 POP 2 X TZIL 25 (60E) 0.4 EMS, DHL 46 POP 1 X LMI 102, 9006 X DHL 186 POP 2, DHL 99 POP 2 X 1368, HP020-5/1(B)/1/7 X HP020-4/1(A)/1/1, DHL 186 POP 2 X DHL 99 POP 2 and DHL 186 POP 2 X 1368-150 GY (119)S.

Usually, it is expected that drought- and low N-tolerant hybrids identified under individual stress environments would also perform well under combined stress environments, however this may not always be the case. Several studies that have explored the interactions between multiple abiotic stresses, have indicated that the combined effects of drought and heat or nutrient deficiency, can lead to yield reductions that are more severe than those observed under individual stresses. Hussain *et al.* (2019) demonstrated that the combined effects of drought and heat stress on maize hybrids resulted in significant reductions in morpho-physiological attributes, yield and nutrient uptake, indicating that the interaction of these stresses is more detrimental than either stress alone. Others reported significant yield losses under combined drought and heat stress that were comparatively higher than the single stresses thereby indicating the importance of understanding how different stresses interact and affect plant productivity (Cohen *et al.*, 2021; Priya *et al.*, 2022; Ru *et al.*, 2024; Zhou *et al.*, 2019).

The comparison of hybrid performance across stress conditions revealed that superiority under a single stress did not necessarily translate to superiority under combined stress conditions. For instance, HP020-4/1(A)/1/1 x DHL 46 POP 1, which ranked highest under drought declined markedly when nitrogen deficiency was introduced, suggesting that drought tolerance alone was insufficient to maintain productivity under compounded stress. Similarly, LMI 102 x HP020-4/1(A)/A/1 was among the best performing hybrids under low N but failed to sustain its advantage under combined drought and low N environment. In contrast, hybrids such as LMI 102 x LMI 140,

LMI 144 x DHL 46 POP 1 and DHL 99 POP 2 x 1368 exhibited stable or improved ranking across environments, implying the presence of complementary physiological traits conferring dual stress resistance. These results are consistent with the findings of Daryanto *et al.* (2016) and Roth *et al.* (2013), who reported that the interaction of water and nitrogen deficits is synergistic rather that additive, often reshaping genotype rankings. Consequently, selection based on single-stress performance may overlook hybrids with integrated adaptive mechanisms, reinforcing the need for multi-environment and multi-trait evaluation approaches such as the BSI to identify genotypes resileint to concurrent stresses.

Under combined drought and low N environment, a wide range of grain yield reduction of 5 to 98%, and an average of 65% was observed. The average grain yield of the top ten single-cross hybrids was higher than Abontem and Obatanpa. Aburo Legon and Pioneer, which were used as checks in this study, were the highest-yielding hybrids under the combined drought and low N environment, outperforming the best single cross hybrid developed, DHL 99 POP 2 X 1368, by 13% and 18 % respectively. These results suggest that the commercial hybrids may be better adapted to the combined stress as compared to the newly developed single cross hybrids which may reflect the cumulative effects of extensive breeding efforts aimed at developing and releasing the commercial hybrids. Most of the inbred lines used in this study are newly developed lines which have not been optimized for stress resilience. Inbred lines are often selected for specific traits, and if those traits do not include drought tolerance or nitrogen use efficiency, the resulting hybrids may not perform well under stress (Rasheed *et al.*, 2017). This is corroborated by Cairns *et al.* (2013) whose findings indicate the importance of selecting for multiple stress tolerance in breeding programmes, as hybrids that are bred under varying environmental conditions perform better under stressful conditions. Using the base index, the top ten superior hybrids selected under

combined drought and low N environment include LMI 144 X DHL 46 POP 1, 1368-150GY (119)S X 9006, HP020-5/1(B)/1/7 X LMI 140, DHL 99 POP 2 X 1368, 9006 X C316-7, DHL 18 POP 1 X LMI 135, LMI 102 X LMI 140, DHL 186 POP 2 X 1368-150GY (119)S, LMI 144 X DHL 18 POP 1 and DHL 46 POP 1 X HP020-5/2(A)/1/7.

Across optimum environments, the best performing hybrid 1368 X DHL 186 POP 2, outperformed the checks, Aburo Legon, Pioneer, Abontem and Obatanpa by 27%, 30%, 33% and 58% respectively. LMI 102 X LMI1 140, which was among the top 10 high yielding hybrids under optimum environments was also identified to be among the top 10 high yielding hybrids under combined drought and low N environment and across low N environments. These results indicate the need for continuous breeding efforts to develop hybrids that are high yielding under optimum environments to replace older varieties especially as the climate continues to change. Abontem and Obatanpa are varieties released over 10 years ago with Aburo Legon being the most recent variety, released by the West Africa Centre for Crop Improvement, University of Ghana in 2017. Pioneer, a foreign hybrid introduced in Ghana, despite its adaptation to the local environment is not easily accessible as it has to be imported to meet its demand in the country hence the need to invest in local research institutions to develop high-yielding varieties which can be easily commercialized by the local seed industry thereby improving accessibility of seeds of improved varieties.

DHL 53 POP 4 X 1368, the highest yielding hybrid across all environments, outperformed the checks Aburo Legon, Pioneer, Abontem and Obatanpa by 25%, 30%, 39% and 61% respectively. LMI 102 X LMI1 140 was also among the top ten high yielding varieties across all environments. Comparatively, plant productivity under the combined drought and low N environment was generally similar to that observed under low N environments. This similarity can be attributed to

several interrelated factors which include physiological responses of plants to nutrient limitations, the interaction between drought and nitrogen availability, and the adaptive strategies utilized by plants under stress conditions. According to He *et al.* (2024), drought stress reducies nitrogen uptake due to restricted soil nitrigen mobility and impaired root functioning, both of which limit plant growth and yield. The interaction between drought and nurtient stress therefore compounds resource limitations, as water scarcity constrains nitrogen transport and assimilation, while nitrogen deficiency in turn restricts photosynthetic efficiency and carbon allocation.

Badu-Apraku *et al.* (2012) reported that under low N environments, plants may exhibit a range of adaptive traits, such as increased root depth and altered leaf morphology, to optimize nutrient uptake. These physiological adaptations can improve plant productivity even when nitrogen is low, as they allow for better resource utilization. However, drought-induced stomatal closure, while conserving water, further limits carbon-dioxide uptake and photosynthetic actvity, aggravating growth reduction. (Shekoofa *et al.*, 2016). The concept of trait plasticity is thus central to understanding how plants respond to varying environments. As highlighted by Kramp *et al.* (2022) and Song *et al.*(2024), plants with high functional plasticity tend to maintain better productivity under combined drought and low N conditions. Consequently, the observed similarity between the two environments in this study suggests that nitrogen limitation, rather than drought, was the more dominant constraint on yield.

One of the importance of trait correlation is the ability to apply indirect selection in breeding programmes. When certain traits are positively correlated with grain yield, breeders are able to indirectly improve yield by selecting for such traits. Under drought, the significant correlation between grain yield and all measured traits except ears per plant and justifies the use of some of these traits, especially anthesis-silking interval and leaf senescence as selection criteria under

drought. These findings are supported by several studies Araus *et al.* (2012), Badu-Apraku *et al.* (2012b), Firezer (2019) and Hao *et al.* (2011) who reported strong correlations between morphological traits such as plant height, ear height and anthesis-silking interval with grain yield, suggesting indirect selection strategies for improving grain yield under drought. Similarly, significant correlations between grain yield and all measured traits across low N environments was observed in this study. Kimutai *et al.* (2021) and Sunday *et al.* (2020) reported significant correlations between grain yield and various traits under low N conditions indicating the potential for selecting traits that can improve yield in low N environments.

In agreement with previous studies on abiotic stress, grain yield under drought, low N and combined drought and low N was moderately and positively associated with grain yield under optimum conditions except for Kwadaso, low N environment (2024, major rainy season) which was negatively correlated with Legon, optimum environment (2023, minor rainy season) (Cairns, 2013). Araus *et al.* (2002) reported that breeding for yield under optimum environments can yield cultivars that perform well not only in optimum environments but also in environments characterized by mild to moderate stress. Grain yield under optimum environments and combined drought and low N environment had a weak positive correlation indicating the presence of independent genetic control of yield in the two environments. Similarly, grain yield under drought had a weak positive correlation with grain yield under low N while grain yield under the combined stress environment was moderately and positively correlated with grain yield under the single stress environment. The moderate relationships between tolerance to abiotic stresses has been reported in wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) (Jagadish *et al.*, 2010; Pinto *et al.*, 2010). However, these results contradict the findings of Cairns (2013) who reported no correlation between drought stress or heat stress with combined heat and drought stress in maize.

A key objective of this study was to identify high-yielding and stable hybrids across drought, low nitrogen (N), combined drought and low N and optimum environments for further testing and commercialization. In sub-Saharan Africa, farmers prefer broadly adapted hybrids due to diverse environmental conditions and seasonal variations, especially in the wake of the unpredictable rainfall patterns caused by climate change. The GGE biplot analysis is a powerful tool for elucidating G x E interactions, allowing for the visual interpretation of genotype performance across multiple environments thereby allowing breeders to identify superior genotypes. This analysis also allows for the identification of mega-environments as demonstrated by (Gungor *et al.*, 2022) in their evaluation of wheat genotypes. In this study, the *"which won where"* biplot was used to select location specific hybrids. The first and second principal component axes (PC1 and PC2) accounted 76% of the total variation in grain yield across environments. This high proportion indicates that the first two principal componenets effectively captured the dffrential performance of hybrids and the pattern of environmental influences on yield. According to W. Yan & Kang (2002), when more than 60% of the variation is explained by the first two PCs, the biplot is considered reliable for interpreting genotype performance and stability patterns.

The "*which-won-where*" polygon view revealed three distinct mega-environments, each characterized by a specific set of winning hybrids and discriminating environments. The hybrid, LMI 102 X LMI 140 (G20), emerged as the top-performing hybrid in environments E2 (Legon OPT, major rainy season 2024) and E6 (Legon LN, major rainy season 2024), reflecting its adaptability to low-N conditions at Legon. Thr grouping of these two sites into one mega-environment suggest similarity in their environmental conditions and selection pressure. In contrast, 1368 x DHL 186 POP 2 (G2) and HP020-4/1(A)/1/1 x DHL 46 POP 1were the winning hybrids in E1 (Legon OPT, minor rainy season 2023) and E3 (Kwadaso OPT, major rainy season

2024), environments associated with relatively favourable conditions. Their positioning on opposite vertices implies specific adaptation rather than broad adaptability (Bambang Priyanto *et al.*, 2024). The third mega environment, consisting of E4 (Legon DT, dry/harmattan season 2023), E5 (Kwadaso LN, major rainy season 2024) and E7 (Legon DTLN, dry season 2023), was associated with TZIL 25 (40C) 0.4 EMS x TXIL 25 25 (60E) 0.4 EMS (G25) as the winning hybrid, indicating that this genotype performs better under a unique combination of environmental factors, likely linked to location-specific stress patterns. The identification of these mega-environments is consistent with the prinicple that genotypes located at the polygon vertices represent those with the greatest yield potential in their respective environmental clusters. Zorić *et al.* (2014) discussed the implications of hybrid positioning within a biplot, recommending that identifying hybrids that perform well under specific stress conditions can help optimize breeding strategies focused on specific locations. Also, in the evaluation of maize hybrids in Western Indonesia, Ruswandi *et al.* (2021b) noted that GGE biplot analysis helps identify hybrids that are not only high-yielding but also stable across multiple environments. The positions of genotypes within the biplot can reveal those that adapt to specific conditions, which is critical when considering hybrid selection for targeted environments (Ruswandi *et al.*, 2021b).

The *"mean vs stability"* biplot identified LMI 102 X LMI 140 (G20) as the highest yielding across all the environments, although it displayed moderate stability across environments. This could be explained by the yield penalties observed leading to significant differences in its yield under stress and optimum environments. In contrast, G3 (1368-150GY(119)S x DHL 184 POP 2), G11 (DHL 99 POP 2 x 9006) and G12 (DHL 99 POP 2 x EXP 124) were identified as the most stable hybrids, combining consistent performance with moderate yields. According to Chaudhary *et al.* (2023) and Mossie *et al.* (2024), ideal genotypes in a GGE biplot combines both high mean performance

and low instability. The results thus highlight LMI 102 X LMI 140 as a promising but environment-sensitive hybrid, while, 1368-150GY(119)S x DHL 184 POP 2, DHL 99 POP 2 x 9006 and DHL 99 POP 2 x EXP 124 may be preferred for wider adaptation due to their stability.

In terms of environmental assessment, the "*discriminativeness vs representativeness*" biplot indicated substantial variability among testing locations. E2, E3 and E6 had long vectors from the origin, suggesting they were the most discriminating environments capable of effectively differentiating among hybrids. Discriminating environments are valuable for identifying high-performing genotypes, although they may not always be ideal for selecting widely adapted ones (Mirosavljević *et al.*, 2018; Ruswandi *et al.*, 2021a; Yamamoto *et al.*, 2021). Conversely, E5 (Kwadaso LN, major rainy season 2024) had the shortest vector, indicating poor discriminating ability and limited usefulness for selection purposes. However, the poximity of E6 (Legon LN, major rainy season 2024) and E3 (Kwadaso OPT, major rainy season 2024) to the AEC abscissa implies they are both discriminating and representative, making them ideal test locations for future breeding evaluations (Aski *et al.*, 2023; C. Liu *et al.*, 2022; Yue *et al.*, 2022).

4.5 Conclusion

Highly significant genotype and G x E effects across all environments indicate exploitable diversity but also pronounced rank chnages among hybrids, justifying multi-environment testing. Moderate to high broad sense heritabality for certain key traits coupled with generally low narrow-sense heritability suggests appreciable total genetic control but also a significant role of dominance/epistasis and G x E. Under combined drought and low N environment, additive gene effects predominated for yield and flowering traits, supporting recurrent selection selection for these traits, while significant SCA for secondary traits supports heterosis exploitation.

The identification of hybrids that excel in specific environments can inform breeders of enhanced strategies aimed at developing climate-resilient varieties and maize varieties developed to specific agronomic conditions. Superior hybrids such as LMI 102 x LMI 140, 1368 x DHL 186 POP 2, HP020-4/1(A)/1/1 X DHL 46 POP 1 and TZIL 25 (40C) 0.4 EMS X TZIL 25 (60E) 0.4 EMS identified under varying environments reflect specific adaptation to distinct environments.

Hybrids such as 1368-150GY(119)S x DHL 184 POP 2, DHL 99 POP 2 x 9006 and DHL 99 POP 2 x EXP 124 were the most stable hybrids hence would serve as good hybrid candidates for broad adaptation. LMI 102 x LMI 140 was identified as the highest yielding hybrid but moderatly stable. These hybrids should be advanced for extensive evaluations to enhance their performance and stability under varying environments.

The identification of superior hybrids under stress environments demonstrates the potential for genetic improvement, however the yield gaps between the tested hybrids and commercial checks under the combined stress environments highlights the need for further testing.This study

highlights the need for continuous breeding efforts to develop hybrids with broad adaptation to multiple environmental conditions

CHAPTER FIVE

5.0 COMBINING ABILITY, GENE ACTION AND HETEROTIC GROUPING OF MAIZE INBRED LINES UNDER VARYING ENVIRONMENTS

5.1 Introduction

Maize production in Ghana is often constrained by drought and low soil fertility, two abiotic stresses that occur simultaneously on farmers' fields. Under optimum conditions, maize requires 500 – 800 mm of well-distributed rainfall per growing season, depending on the variety and soil type, with optimal soil moisture conditions between 60 – 80% of field capacity for maximum prodictivity (Suriadi *et al.*, 2024; Zizinga *et al.*, 2022). However, the erratic rainfall patterns increasingly experienced by farmers, coupled with widespread low soil nitrogen levels, have led to severe yield reductions in maize. Consequently, breeding for high-yielding hybrids with resilience to the individual stresses as well as combined stresses is integral to increased productivity of maize in the country.

Crucial to the development of high-yielding hybrids especially under varying environments such as drought and low soil nitrogen is the understanding of combining ability and heterotic grouping of maize inbred lines. Recent studies have highlighted the significance of both general combining ability (GCA) and specific combining ability (SCA) in assessing the performance of maize inbred lines across varying stress conditions (Oyetunde *et al.*, 2020; Kumar *et al.*, 2022; Maazou *et al.*, 2023; Arora *et al.*, 2024; Kamara *et al.*, 2024).

A key strategy in breeding for resilience is identifying parental inbred lines that combine well to produce superior hybrids under stress conditions while understanding the genetic basis for hybrid performance. Banoth *et al.* (2021) emphasized that placing inbred lines in well-defined heterotic

groups increases the probability of success in heterosis breeding, particularly under stress environments. Similarly, Perić *et al.* (2021) explored the use of genetic distances among maize inbred lines to predict heterosis and combining ability, underscoring the importance of understanding genetic relationships for effective hybrid development.

In environments where stress factors like drought and low nitrogen are prevalent, the need to identify inbred lines with favourable combining ability is even more critical. Studies by Osuman *et al.* (2022) and Nasser *et al.* (2020a) reported that hybrid performance under combined drought and heat stress was strongly infleuced by the nature of gene action and parental compatability.

Understanding the type of gene action governing the inheritance of important agronomic traits under contrasting environments is vital for identifying promising inbred lines and for guiding appropriate breeding strategies aimed at develop tolerant hybrids for multiple stresses (Badu-Apraku *et al.*, 2016). Several studies have highlighted the mode of gene action of traits under varying environmental conditions (Abu *et al.*, 2021; Bhadmus *et al.*, 2021; Ertiro *et al.*, 2017; Sunday *et al.*, 2020). According to Badu-Apraku *et al.* (2020), understanding the genetic architecture of traits under different environmental conditions enables breeders to select appropriate inbred lines that can contribute to the development of hybrids with enhanced tolerance to multiple stresses. This is relevant, especially, in the face of climate change and continuous prevalence of abiotic stresses in agricultural systems.

The objectives of this study were to:

(i) estimate the general and specific combining abilities of selected maize inbred lines under drought stress, low-nitrogen, combined drought and low-nitrogen and optimum moisture and nitrogen conditions,

(ii) determine the nature and magnitude of gene action controlling grain yield and other agronomic traits among the selected lines under these contrasting environments, and

(iii) group the inbred lines into heterotic groups using the heterotic grouping method based on the combining ability of multiple traits (HGCAMT).

5.2 Materials and Methods

The development of the hybrids was done as described in Chapter 4 of this thesis, section 4.2.1. Field evaluation across the multiple locations and varying treatments, that is drought, low N, combined drought and low N, and optimal environments was done as described in Chapter 4 of this thesis, section 4.2.2. The management of the trials under drought and optimal environments as well as data collection relevant for this study were done according to the methods described in Chapter 3 of this thesis, section 3.2.4 to section 3.2.6.

5.3 Data analyses

5.3.1 Combining ability effects

A mixed model analysis by REML was used in estimating these effects where GCA and SCA were treated as random factors in R so that the best linear unbiased predictions (BLUPs) was obtained for the parental (GCA) and hybrid interaction (SCA) effects (De La Vega & Chapman, 2006; Luz *et al.*, 2024). The model used was as follows:

$$Y_{ijkm} = \mu + GCA_i + GCA_j + SCA_{ij} + R_k + B_{m(k)} + \varepsilon_{ijkm}$$

where: Y_{ijkm} is the observation of the hybrid resulting from parents *i* and *j*; μ is the overall mean; GCA_i and GCA_j are the general combining ability effects of the two parents forming the hybrid; SCA_{ij} is the specific combining ability effect for the cross between parents *i* and *j*; R_k represents

the random replication effect; $B_{m(k)}$ represents the random block effect nested within replications and ε_{ijkm} is the residual error.

5.3.2 Heterotic groupings of inbred lines

Inbred lines were assigned to heterotic groups under drought stress, low soil N, combined drought and low soil nitrogen, optimal environments and across all environments based on GCA of multiple traits (HGCAMT) method using the statistical model defined as follows (Badu-Apraku, Fakorede, Gedil, *et al.*, 2016b):

$$Y = \sum_{i=1}^{n} \left(\frac{(Y_i - y_i)}{s}\right) + \varepsilon_{ij}$$

where: Y is HGCAMT representing the genetic value that measures relationship among the genotypes based on the GCA of multiple traits from *i* to *n;* Y_i is the individual GCA effect of genotypes for trait *i;* y_i is the mean of GCA effects across genotypes for trait *i;* *s* represents the standard deviation of the GCA effects of trait *i* and ε_{ij} is the residual of the model associated with the combination of the inbred *i* and trait *j*.

For each environment, the individual GCA effects of inbreds evaluated as female and male parents were averaged to obtain a combined estimate that reflects their overall general combining ability. The GCA values for all traits were standardized to remove scale effects and align trait directions. The resulting standardized matrix was subjected to multivariate hierarchical cluster analysis using the Ward's D^2 linkage method, which groups inbreds based on their multivariate GCA similarity profiles.

The optimal number of clusters (K) in the data was determined using the Elbow method and used to perform the hierarchical grouping of the inbred lines (Obeng-Bio *et al.*, 2019a). This was achieved by plotting the within-cluster sum of squares (WSS) against increasing cluster numbers and identifying the inflection point ("elbow"). The selected K value was then applied to define the final cluster membership of inbreds under each environment.

The analysis was implemented in R (Version 2025.09.1+401) using custom scripts where functions from the packages lme4, cluster and stats were used for model fitting, distance computation, and hierarchical clustering.

5.4 Results

5.4.1 General Combining Ability (GCA) effects for grain yield and associated traits

Under drought environment, the GCA-female effects for grain yield ranged from -41.38 for 1368 to 66.04 for DHL 99 POP 2, with several inbred lines such as DHL 99 POP 2, DHL 184 POP 2, DHL 142 POP 1 and LMI 102 showing positive GCA effects, signifying their importance as good general combiners for yield. Negative GCA effects recorded for lines such as 1368, 9006, C316-7 and TZIL 25 (43A) 0.4 EMS suggest limited additive contribution to grain yield as female parental lines. In contrast, the GCA-male effects were very small in magnitude, ranging between -2.9×10^{-6} and 4.3×10^{-6}, indicating minimal variation attributable to the male inbred lines for yield under this environment (Table 5.1). Variation among inbred lines for plant height, days to anthesis, days to silking, leaf senescence (LS) and chlorophyll content (SPAD) was observed. Inbred lines such as LMI 102, DHL 46 POP 1 and LMI 140 showed positive GCA effects for plant height. HP020-5/2(A)/1/7, LMI 140 and DHL 142 POP 1 showed negative GCA effcets for flwoering traits indicating earliness, whereas HP020-4/1(A)/1/1 and DHL 46 POP 1 showed positive GCA effects indicating late maturity. Leaf senescence and SPAD readings showed generally small effects, but DHL 142 POP 1 and HP020-4/1(A)/1/1 displayed higher chlorophyll content and delayed leaf senescence.

Significant differences in GCA effects were observed among the 24 inbred lines evaluated under low-nitrogen environments at Kwadaso and Legon. At both locations, female GCA effects for grain yield ranged from -81.23 to 122.18 (Table 5.2 and Table 5.3). Inbred lines such as DHL 186 POP 2, DHL 184 POP 2, LMI 102 and LMI 140 consistently exhibited positive GCA vlaues, identifying them as superior general combiners for yield. In contrast, DHL 18 POP 1, 9006 and TZIL 25 mutant lines, displayed negative GCA effects, indicating limit additive contribution to

Table 5. 1: General combining abilities of selected traits of the 24 inbreds under DT environment, Legon (2023) in Ghana.

Trait	YLD		PH		DYANTH		DYSK		LS		SPAD	
Inbred	**GCA-F**	**GCA-M**	**GCA-F**	**GCA-M**	**GCA-F**	**GCA-M**	**GCA-F**	**GCA-M**	**GCA-F**	**GCA-M**	**GCA-F**	**GCA-M**
1368	-41.38	-8.88E-07	-2.36	1.26	0.62	1.16	0.38	0.54	0.02	0.07	-0.35	0.41
9006	-37.55	1.03E-06	-6.26	-0.97	0.66	0.82	-0.18	0.10	0.05	-0.02	1.35	0.45
1368-150GY(119)S	9.52	-2.39E-06	-3.30	-2.15	0.99	0.69	2.04	0.67	0.05	0.25	0.44	0.75
C316-7	-26.06	-2.93E-06	-5.38	-1.89	0.87	-0.52	0.88	0.28	0.00	0.35	-1.41	0.43
DHL 142 POP 1	25.00	8.62E-07	-0.99	-0.58	-1.92	-0.88	-1.84	-0.44	-0.04	-0.28	2.18	0.27
DHL 18 POP 1	-14.61	7.37E-07	-1.13	-0.77	-0.75	-1.11	-0.23	-0.53	0.02	0.09	-1.70	0.12
DHL 184 POP 2	27.68	1.13E-07	-3.22	-0.51	-1.87	-2.12*	-2.00	-0.90	-0.03	0.07	1.06	-0.42
DHL 186 POP 2	21.21	-1.84E-07	7.58	-1.33	-1.89	-0.42	-2.26	-0.82	-0.02	-0.13	-1.07	0.08
DHL 46 POP 1	13.30	8.17E-07	9.24	2.10	0.81	0.52	0.61	0.41	-0.05	0.13	0.56	-0.10
DHL 53 POP 4	-28.51	1.25E-07	2.90	3.07	1.19	-0.66	2.08	-0.23	-0.02	-0.12	-1.85	-0.62
DHL 99 POP 2	66.04	4.26E-06	0.11	-0.03	-0.78	-0.55	-2.15	-0.83	-0.07	-0.20	1.34	-0.63
EXP 124	-1.29	1.26E-06	-3.08	1.69	1.42	0.66	0.84	0.94	-0.02	-0.24	0.99	-0.29
HP020-4/1(A)/1/1	39.95	1.16E-07	-4.51	-0.11	2.50*	1.30	0.88	0.46	-0.05	-0.15	1.99	-0.59
HP020-5/1(B)/1/7	14.78	-1.53E-06	2.05	-0.27	2.08	0.96	1.17	0.55	0.01	0.06	1.37	0.14
HP020-5/2(A)/1/7	7.77	5.08E-07	3.74	0.06	-2.48*	-1.45	-3.23*	-0.75	0.04	0.04	-2.85	0.11
HP020-6/2(9)/9/6	-9.25	-2.93E-06	-3.79	-1.00	1.66	1.32	2.28	0.88	0.00	0.34	0.24	-0.19
LMI 102	33.68	-1.20E-06	12.97*	-0.36	-0.85	0.74	-0.72	0.79	-0.03	-0.17	-0.92	-0.18
LMI 135	5.35	3.37E-06	-5.32	1.55	-0.38	-0.37	-0.80	-0.32	-0.02	-0.47	0.26	0.25
LMI 140	10.10	3.42E-06	7.12	5.70	-2.34*	-1.62	-2.35	-1.72	0.01	-0.13	-0.10	-0.04
LMI 141	-21.41	6.94E-09	-0.90	-1.92	-0.29	-0.03	1.73	0.37	0.03	0.09	-1.17	0.30
LMI 144	-35.14	1.32E-06	-2.57	0.30	-0.11	-0.65	0.65	-0.33	0.06	0.19	-0.31	-0.21
TZIL 25 (40C) 0.4 EMS	-1.33	-1.08E-06	-5.96	-0.49	-0.01	0.15	0.35	-0.03	0.04	0.19	-0.31	0.22
TZIL 25 (43A) 0.4 EMS	-30.00	5.18E-08	-2.21	-0.68	1.40	1.29	1.84	0.24	0.02	-0.26	-0.41	0.03
TZIL 25 (60E) 0.4 EMS	-27.87	-4.85E-06	5.25	-2.65	-0.53	0.75	0.03	0.67	0.03	0.27	0.67	-0.30
S.E	**104.06**	**0.03**	**5.84**	**3.83**	**1.03**	**0.93**	**1.36**	**1.01**	**0.14**	**0.30**	**1.80**	**1.04**

**, significant at 0.05 probability level; YLD, grain yield (kg/ha); PH, plant height; DYANTH, days to 50% anthesis; DYSK, days to 50% silking; LS, leaf senescence; SPAD, leaf chlorophyll content; GCA-f, general combining ability effects of female inbred parent; GCA-m, general combining ability effects of male inbred parent; SE, standard error.*

yield. Between sites, male GCA effects were highly variable but averaged to zero across sites, suggesting that male contributions to yield were environment-specific and influenced by genotyope x environment interactions (Table 5.4). For flowering traits, significant variation was also observed. Negative GCA effects for days to 50% anthesis in HP020-5/2(A)/1/7, DHL 184 POP 2 and LMI 140 indicated their potential for early maturity, which is an advantage under stress conditions. GCA effects for physiological traits such as leaf senescence and SPAD readings were small though DHL 142 POP 1, DHL 186 POP 2 and LMI 102 displayed postive GCA values, suggesting better cholorophyll retention and stronger stay-green characteristics under nitrogen stress.

Under DTLN environment, the female and male GCA effects for grain yield were generally small for all the inbred lines (Table 5.5). This indicated limited addtitve contribution to yield of the inbred lines under the combined stress. However inbred lines such as DHL 186 POP 2, DHL 46 POP 1, DHL 99 POP 2 and HP020-5/1(B)/7 recorded slightly positive GCA values whereas 9006, HP020-5/2(A)/1/7 and TZIL 25 mutant lines showed negative effects. Significant differences was observed for plant and ear height, with female GCA effects ranging from -21.20 for 9006 to 17.3 for LMI 102. For flowering traits, negative GCA values for C316-7, DHL 184 POP 2 and HP020-5/2(A)/1/7 indicated earlier anthesis and silking, whereas DHL 46 POP 1, LMI 102 and HP020-5/1(B)/1/7 indicated late flowering. Low to moderate GCA effects was recorded for leaf senescence (LS) with positive values for DHL 186 POP 2 and 9006 suggesting delayed leaf drying.

Table 5. 2: General combining abilities of selected traits of the 24 inbreds under LN environment (2024), Kwadaso, environment in Ghana.

	YLD		DYANTH		ASI		LS		SPAD		STGR	
Inbred	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**
1368	-21.71	-24.41	0.09	0.78	0.01	0.02	0.02	0.07	-0.24	-0.01	0	0.08
9006	-74.50	1.72	1.13	1.04	-0.03	-0.01	0.00	0.03	-0.22	0.08	0	0.12
1368-150GY(119)S	-6.77	21.02	1.16	0.46	0.04	0.02	0.01	0.07	-0.18	0.10	0	0.14
C316-7	24.31	-163.94	0.98	1.82	0.00	0.01	0.04	-0.03	-0.21	-0.21	0	0.08
DHL 142 POP 1	19.63	106.91	-0.71	-1.46	0.00	0.00	-0.05	-0.07	0.99	0.40	0	-0.16
DHL 18 POP 1	-81.23	-53.30	-0.95	-0.68	-0.02	-0.02	0.01	-0.02	-0.18	0.11	0	0.12
DHL 184 POP 2	75.09	11.37	-1.55*	-0.46	0.05	0.01	-0.06	0.03	0.03	-0.43	0	-0.02
DHL 186 POP 2	122.18	280.76	-0.81	-2.14*	-0.04	-0.04	-0.02	-0.03	1.25	0.44	0	-0.11
DHL 46 POP 1	39.17	118.16	0.28	-0.09	0.02	0.02	-0.05	0.07	0.73	-0.21	0	-0.12
DHL 53 POP 4	-8.79	59.83	1.10	0.46	0.00	-0.01	-0.01	-0.02	-0.57	-0.57	0	-0.12
DHL 99 POP 2	87.96	162.92	-1.26	-1.26	-0.02	-0.03	0.03	0.06	-0.41	0.09	0	-0.01
EXP 124	-46.77	-110.96	1.18	1.29	-0.02	0.02	-0.01	-0.06	0.04	-0.17	0	0.00
HP020-4/1(A)/1/1	-23.64	27.50	1.27	-0.05	0.06	-0.03	-0.02	0.01	0.34	0.17	0	-0.05
HP020-5/1(B)/1/7	-41.15	25.91	1.67*	0.01	0.01	0.04	0.01	-0.02	-0.56	0.05	0	-0.15
HP020-5/2(A)/1/7	40.84	-10.04	-1.67*	-1.21	-0.04	0.02	0.00	-0.07	-0.94	-0.14	0	-0.01
HP020-6/2(9)/9/6	-60.53	-73.98	0.95	0.87	-0.01	0.02	0.01	0.05	0.01	-0.04	0	0.08
LMI 102	94.36	0.38	-0.52	-0.66	0.03	0.01	0.01	-0.03	0.95	0.23	0	-0.15
LMI 135	19.46	-42.84	-1.17	0.62	0.01	-0.01	0.04	-0.03	0.09	0.66	0	-0.01
LMI 140	65.72	-79.88	-2.52**	-0.10	0.01	-0.02	0.02	0.03	0.31	0.13	0	0.04
LMI 141	-28.76	-44.23	0.37	-0.29	0.02	0.01	-0.01	0.00	-0.04	-0.26	0	0.04
LMI 144	-20.61	-64.80	-0.37	-1.57	-0.03	0.02	0.02	0.06	-0.08	-0.29	0	-0.01
TZIL 25 (40C) 0.4 EMS	-62.53	-45.93	0.86	0.55	-0.04	-0.02	0.01	-0.03	-0.45	-0.41	0	0.07
TZIL 25 (43A) 0.4 EMS	-69.91	-16.07	-0.20	1.00	-0.02	0.00	0.00	-0.01	-0.74	-0.03	0	0.09
TZIL 25 (60E) 0.4 EMS	-41.81	-86.10	0.72	1.09	0.03	-0.02	0.01	-0.07	0.11	0.30	0	0.07
S.E	**98.07**	**162.38**	**0.76**	**1.01**	**0.18**	**0.11**	**0.10**	**0.13**	**1.01**	**0.77**	**0**	**0.14**

***, significant at 0.001 probability level; *, significant at 0.05; YLD, grain yield (kg/ha); ASI, anthesis-silking interval; DYANTH, days to anthesis; LS, leaf senescence; SPAD, leaf chlorophyll content; STGR, stay-green characteristic, SE, standard error; GCA-f, general combining ability effects of female inbred parent; GCA-m, general combining ability effects of male inbred parent.*

Table 5. 3: General combining abilities of selected traits of the 24 inbreds under LN environment (2024), Legon, environment in Ghana.

	YLD		DYANTH		ASI		LS		SPAD		STGR	
Inbred	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**
1368	-21.71	-145.83	0.09	1.13	-0.05	0.02	0.02	0.07	-0.24	-0.01	0	0.05
9006	-74.50	45.23	1.13	-0.07	-0.04	-0.01	0.00	0.03	-0.22	0.08	0	-0.07
1368-150GY(119)S	-6.77	-10.34	1.16	-0.24	0.04	0.03	0.01	0.07	-0.18	0.10	0	-0.11
C316-7	24.31	-82.71	0.98	0.66	0.00	0.00	0.04	-0.03	-0.21	-0.21	0	0.00
DHL 142 POP 1	19.63	-37.82	-0.71	-1.21	0.01	0.00	-0.05	-0.07	0.99	0.40	0	-0.04
DHL 18 POP 1	-81.23	242.29	-0.95	-1.15	0.08	-0.02	0.01	-0.02	-0.18	0.11	0	-0.06
DHL 184 POP 2	75.09	112.60	-1.55*	-1.58	-0.06	0.01	-0.06	0.03	0.03	-0.43	0	-0.05
DHL 186 POP 2	122.18	-111.26	-0.81	-0.61	0.00	-0.04	-0.02	-0.03	1.25	0.44	0	0.00
DHL 46 POP 1	39.17	-120.74	0.28	1.55	-0.02	0.02	-0.05	0.07	0.73	-0.21	0	0.04
DHL 53 POP 4	-8.79	-156.34	1.10	0.26	0.04	-0.01	-0.01	-0.02	-0.57	-0.57	0	-0.10
DHL 99 POP 2	87.96	-63.64	-1.26	-0.59	-0.07	-0.03	0.03	0.06	-0.41	0.09	0	0.01
EXP 124	-46.77	216.24	1.18	0.03	0.01	0.02	-0.01	-0.06	0.04	-0.17	0	0.05
HP020-4/1(A)/1/1	-23.64	350.53	1.27	1.18	-0.02	-0.03	-0.02	0.01	0.34	0.17	0	-0.08
HP020-5/1(B)/1/7	-41.15	-268.40	1.67*	1.38	-0.08	0.04	0.01	-0.02	-0.56	0.05	0	0.15
HP020-5/2(A)/1/7	40.84	-104.18	-1.67*	-0.70	-0.01	0.02	0.00	-0.07	-0.94	-0.14	0	0.09
HP020-6/2(9)/9/6	-60.53	-206.81	0.95	0.60	0.09	0.02	0.01	0.05	0.01	-0.04	0	0.08
LMI 102	94.36	205.83	-0.52	-0.36	0.04	0.01	0.01	-0.03	0.95	0.23	0	-0.05
LMI 135	19.46	-94.07	-1.17	0.39	-0.03	-0.01	0.04	-0.03	0.09	0.66	0	0.11
LMI 140	65.72	357.97	-2.52**	-0.79	-0.05	-0.03	0.02	0.03	0.31	0.13	0	0.01
LMI 141	-28.76	-96.29	0.37	0.20	0.06	0.01	-0.01	0.00	-0.04	-0.26	0	0.17
LMI 144	-20.61	-57.55	-0.37	-0.72	0.00	0.02	0.02	0.06	-0.08	-0.29	0	-0.02
TZIL 25 (40C) 0.4 EMS	-62.53	-66.07	0.86	-0.83	0.03	-0.02	0.01	-0.03	-0.45	-0.41	0	0.00
TZIL 25 (43A) 0.4 EMS	-69.91	-29.95	-0.20	0.61	0.01	0.00	0.00	-0.01	-0.74	-0.03	0	-0.06
TZIL 25 (60E) 0.4 EMS	-41.81	121.30	0.72	0.87	0.03	-0.02	0.01	-0.07	0.11	0.30	0	-0.12
S.E	**98.07**	**162.38**	**0.76**	**1.01**	**0.18**	**0.11**	**0.10**	**0.13**	**1.01**	**0.77**	**0**	**0.14**

***, significant at 0.001 probability level; *, significant at 0.05; YLD, grain yield (kg/ha); ASI, anthesis-silking interval; DYANTH, days to anthesis; LS, leaf senescence; SPAD, leaf chlorophyll content; STGR, stay-green characteristic, SE, standard error; GCA-f, general combining ability effects of female inbred parent; GCA-m, general combining ability effects of male inbred parent.*

Table 5. 4: General combining abilities of selected traits of the 24 inbreds across LN environments, Legon and Kwadaso, in Ghana.

	YLD		PH		DYANTH		ASI		LS		SPAD	
Inbred	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**
1368	-21.71	0	0.19	-0.99	0.09	0.61	0	0.02	0.02	0.07	-0.24	-0.01
9006	-74.50	0	-0.36	-4.61	1.13	0.31	0	-0.01	0.00	0.03	-0.22	0.08
1368-150GY(119)S	-6.77	0	-0.98	-0.94	1.16	0.07	0	0.02	0.01	0.07	-0.18	0.10
C316-7	24.31	0	-1.51	-2.87	0.98	0.80	0	0.01	0.04	-0.03	-0.21	-0.21
DHL 142 POP 1	19.63	0	0.18	2.32	-0.71	-0.86	0	0.00	-0.05	-0.07	0.99	0.40
DHL 18 POP 1	-81.23	0	-0.29	-1.90	-0.95	-0.59	0	-0.02	0.01	-0.02	-0.18	0.11
DHL 184 POP 2	75.09	0	-0.63	1.04	-1.55	-0.66	0	0.01	-0.06	0.03	0.03	-0.43
DHL 186 POP 2	122.18	0	2.76	1.97	-0.81	-0.88	0	-0.04	-0.02	-0.03	1.25	0.44
DHL 46 POP 1	39.17	0	1.14	1.78	0.28	0.47	0	0.02	-0.05	0.07	0.73	-0.21
DHL 53 POP 4	-8.79	0	-0.48	3.27	1.10	0.23	0	-0.01	-0.01	-0.02	-0.57	-0.57
DHL 99 POP 2	87.96	0	0.23	0.57	-1.26	-0.60	0	-0.03	0.03	0.06	-0.41	0.09
EXP 124	-46.77	0	-1.18	-1.68	1.18	0.43	0	0.02	-0.01	-0.06	0.04	-0.17
HP020-4/1(A)/1/1	-23.64	0	-1.75	5.31	1.27	0.37	0	-0.03	-0.02	0.01	0.34	0.17
HP020-5/1(B)/1/7	-41.15	0	-0.76	-0.15	1.67*	0.44	0	0.04	0.01	-0.02	-0.56	0.05
HP020-5/2(A)/1/7	40.84	0	0.03	-1.14	-1.67*	-0.61	0	0.02	0.00	-0.07	-0.94	-0.14
HP020-6/2(9)/9/6	-60.53	0	-0.26	0.76	0.95	0.47	0	0.01	0.01	0.05	0.01	-0.04
LMI 102	94.36	0	4.02	3.61	-0.52	-0.33	0	0.01	0.01	-0.03	0.95	0.23
LMI 135	19.46	0	0.08	-0.61	-1.17	0.32	0	-0.01	0.04	-0.03	0.09	0.66
LMI 140	65.72	0	3.08	1.04	-2.52**	-0.29	0	-0.02	0.02	0.03	0.31	0.13
LMI 141	-28.76	0	-0.71	-0.68	0.37	-0.03	0	0.01	-0.01	0.00	-0.04	-0.26
LMI 144	-20.61	0	0.73	1.73	-0.37	-0.73	0	0.02	0.02	0.06	-0.08	-0.29
TZIL 25 (40C) 0.4 EMS	-62.53	0	-1.39	0.64	0.86	-0.09	0	-0.02	0.01	-0.03	-0.45	-0.41
TZIL 25 (43A) 0.4 EMS	-69.91	0	-0.53	-4.10	-0.20	0.52	0	0.00	0.00	-0.01	-0.74	-0.03
TZIL 25 (60E) 0.4 EMS	-41.81	0	-1.61	-4.39	0.72	0.63	0	-0.02	0.01	-0.07	0.11	0.30
S.E	**98.07**	**0**	**3.12**	**4.44**	**0.76**	**0.68**	**0**	**0.11**	**0.10**	**0.13**	**1.01**	**0.77**

***, significant at 0.001 probability level; *, significant at 0.05; YLD, grain yield (kg/ha); ASI, anthesis-silking interval; DYANTH, days to anthesis; LS, leaf senescence; SPAD, leaf chlorophyll content; STGR, stay-green characteristic, SE, standard error; GCA-f, general combining ability effects of female inbred parent; GCA-m, general combining ability effects of male inbred parent.*

Substantial variation was observed in the GCA effects for grain yield under optimal conditions at Legon and Kwadaso (Table 5.6). Female GCA effects were generally low for all the inbred lines while male GCA effects were large and variable. At Legon, in 2023, during the minor rainy season, high positive male GCA effects for grain yield were recorded for 1368, DHL 186 POP 2, LMI 140 and LMI 141 while at the same location, in 2024, during the major rainy season, high positive male GCA effects were recorded for DHL 186 POP 2, DHL 53 POP 4, EXP 124, LMI 135 and LMI 140. At Kwadaso, in 2024, during the major rainy season, high positive male GCA effects for grain yield were recorded for DHL 184 POP 2, DHL 46 POP 1, DHL 99 POP 2, LMI 135 and LMI 140 (Table 5.6). Across sites, male GCA effects for were large and variable ranging from -239.32 to 154.16. LMI 140, LMI 135 and LMI 141 displayed high positive male GCA effects whereas TZIL 25 (43A) 0.4 EMS and 9006 showed high negative male GCA effects (Table 5.7). The overall female GCA effects across sites remained small whereas male GCA effects were larger but environment-specific.

Across all environments, substantial variability in GCA effects was observed among the 24 inbred lines (Table 5.8). Female GCA effects for yield ranged from -70.59 to 92.15, with DHL 99 POP 2, LMI 102, LMI 135 and DHL 186 POP 2, exhibiting the highest postive effects, indicating superior additive potential across contrasting environments. Male GCA effects for yield were also highly variable ranging from -94.33 to 91.57 with LMI 140, LMI 135 and DHL 186 POP 2 emerging as strong general combiners across environments. For plant and ear height, positive female GCA effects were observed for DHL 186 POP 2, DHL 46 POP 1, LMI 135 and LMI 141. Flowering traits displayed narrower GCA ranges though inbreds such as DHL 184 POP 2, DHL 186 POP 2 and LMI 140 showing small negative GCA effects indicating a tendency

Table 5. 5: General combining abilities of selected traits of the 24 inbreds under DTLN (2023) environment in Ghana.

	YLD		PH		EH		DYANTH		DYSK		LS	
INBRED	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**
1368	-0.0001	-5.36E-08	-4.19	-9.37	-3.78	-6.41	1.48	1.38	1.85	2.92	-0.04	0.26
9006	-0.0002	8.27E-07	-21.20*	-1.76	-13.06**	-1.66	2.03	0.64	1.77	-0.12	-0.01	0.44
1368-150GY(119)S	0.0001	-5.76E-07	-9.71	-3.28	-7.14	-3.43	1.66	1.82	1.64	1.49	-0.25	0.19
C316-7	0.0000	-6.09E-07	-12.23	-4.04	-8.85*	-5.43	2.00	0.03	1.31	0.78	0.35	-0.68
DHL 142 POP 1	0.0002	7.04E-07	-0.22	-0.52	2.24	-5.88	-1.47	-0.60	-0.87	0.09	-0.44	-0.56
DHL 18 POP 1	0.0000	1.58E-06	-0.77	-0.09	5.30	4.72	-1.41	-3.32*	-1.59	-4.85*	0.16	-0.01
DHL 184 POP 2	0.0002	5.24E-07	-3.35	-0.05	-6.37	-3.61	-1.52	-1.70	-1.42	-1.11	-0.36	-0.36
DHL 186 POP 2	0.0003	1.28E-06	13.73*	3.47	9.05	2.82	-1.85	-1.04	-3.44*	-2.13	0.00	0.28
DHL 46 POP 1	0.0001	1.67E-06	14.31*	6.95	11.90	11.34*	0.31	0.35	-0.39	-0.25	0.08	0.16
DHL 53 POP 4	-0.0002	5.69E-07	-6.70	8.69	-0.53	7.74*	1.69	-0.52	3.39*	-0.47	-0.14	0.26
DHL 99 POP 2	0.0003	6.53E-07	1.01	-4.68	1.60	-4.23	-1.93	-0.80	-3.14*	-1.73	0.33	-0.08
EXP 124	0.0000	-8.78E-07	3.14	2.44	6.06	4.04	0.77	0.95	-0.35	1.73	0.01	-0.18
HP020-4/1(A)/1/1	0.0001	-1.30E-06	-2.86	1.22	-4.68	-4.02	2.48*	1.68	1.64	0.41	-0.14	-0.30
HP020-5/1(B)/1/7	0.0000	-1.41E-06	4.23	2.26	1.92	1.71	3.84**	1.23	2.62	1.45	-0.09	0.42
HP020-5/2(A)/1/7	-0.0002	2.56E-06	-4.44	2.25	1.41	5.29	-1.90	-2.47*	-2.15	-3.28*	0.36	-0.23
HP020-6/2(9)/9/6	-0.0002	-1.49E-06	0.25	1.30	-6.23	-2.30	0.87	1.58	1.47	1.90	0.26	0.06
LMI 102	-0.0001	-9.38E-07	17.13*	2.58	7.24	1.54	-0.65	0.10	0.66	0.72	0.11	0.04
LMI 135	0.0001	6.24E-07	0.51	-1.31	-1.66	1.20	-1.25	-0.14	-2.04	-0.17	-0.16	-0.13
LMI 140	0.0000	2.60E-06	10.12	10.04	7.57	9.91	-2.99	-1.75	-2.68	-2.55	0.02	-0.05
LMI 141	0.0000	-2.00E-06	3.29	-2.50	3.73	0.35	-1.41	-0.01	-1.33	1.47	0.04	0.52
LMI 144	0.0002	-1.25E-06	7.42	-1.35	4.07	0.33	-0.74	0.11	-0.98	-0.17	-0.14	0.15
TZIL 25 (40C) 0.4 EMS	-0.0001	-9.07E-07	-2.87	-6.57	-4.18	-5.12	-0.01	2.93*	1.21	2.14	-0.07	-0.24
TZIL 25 (43A) 0.4 EMS	-0.0002	-1.15E-06	-1.44	-3.73	-1.34	-4.91	-0.28	0.16	1.03	0.92	-0.02	0.01
TZIL 25 (60E) 0.4 EMS	-0.0001	-1.03E-06	-5.17	-1.93	-4.29	-3.99	0.28	-0.62	1.79	0.81	0.15	0.04
S.E	**0.2030**	**0.0197712**	**6.80**	**5.54**	**3.90**	**3.69**	**1.08**	**1.03**	**1.45**	**1.42**	**0.27**	**0.31**

, significant at 0.05 probability level;, significant at 0.01 probability level; YLD, grain yield (kg/ha); 100-KW, 100 kernel weight (g); DYANTH, days to anthesis; DYSK, days to silking; EH, ear height; LS, leaf senescence; GCA-f, general combining ability effects of female inbred parent; GCA-m, general combining ability effects of male inbred parent; SE, standard error*

Table 5. 6: Yield General Combining Abilities (GCA) of the 24 inbreds under optimal environments, Legon and Kwadaso, in Ghana.

	LG23		LG24		KW24	
INBRED	**YLD_F**	**YLD_M**	**YLD_F**	**YLD_M**	**YLD_F**	**YLD_M**
1368	8.76E-06	166.48	7.80E-06	-139.73	7.63E-06	44.08
9006	-1.85E-05	-188.80	-1.82E-05	-120.22	-1.89E-05	-182.31
1368-150GY(119)S	8.68E-06	-46.36	8.58E-06	-159.66	7.94E-06	-54.07
C316-7	-1.24E-05	-97.97	-1.37E-05	6.29	-1.34E-05	-191.62
DHL 142 POP 1	1.12E-05	24.19	1.13E-05	-146.06	1.24E-05	-193.26
DHL 18 POP 1	9.12E-06	28.77	9.34E-06	17.77	9.36E-06	2.38
DHL 184 POP 2	-1.16E-05	89.83	-1.15E-05	92.95	-1.12E-05	135.03
DHL 186 POP 2	7.99E-06	130.77	8.18E-06	171.78	8.21E-06	7.58
DHL 46 POP 1	-2.27E-06	-96.65	-1.87E-06	65.57	-1.76E-06	259.40
DHL 53 POP 4	-1.14E-05	85.13	-1.12E-05	152.46	-1.09E-05	29.85
DHL 99 POP 2	1.90E-05	82.70	1.88E-05	-112.11	1.91E-05	140.76
EXP 124	-1.31E-05	35.32	-1.19E-05	194.73	-1.29E-05	70.33
HP020-4/1(A)/1/1	-4.02E-06	87.27	-4.08E-06	65.48	-4.14E-06	16.43
HP020-5/1(B)/1/7	-3.23E-06	5.95	-3.06E-06	21.54	-3.80E-06	32.92
HP020-5/2(A)/1/7	4.07E-06	-90.28	4.60E-06	-164.71	4.12E-06	-14.05
HP020-6/2(9)/9/6	-7.65E-06	-44.96	-8.09E-06	-10.25	-8.43E-06	-64.84
LMI 102	7.25E-06	99.17	7.82E-06	28.81	7.54E-06	74.34
LMI 135	2.26E-05	2.81	2.24E-05	187.57	2.25E-05	192.80
LMI 140	7.97E-06	237.89	7.38E-06	257.69	8.06E-06	150.43
LMI 141	1.50E-06	122.90	1.63E-06	251.01	1.26E-06	77.73
LMI 144	-1.02E-05	-153.12	-9.57E-06	-16.09	-9.51E-06	-27.34
TZIL 25 (40C) 0.4 EMS	-1.91E-05	-40.77	-1.96E-05	-92.37	-1.89E-05	-85.97
TZIL 25 (43A) 0.4 EMS	1.56E-05	-357.96	1.56E-05	-309.29	1.61E-05	-335.59
TZIL 25 (60E) 0.4 EMS	-1.04E-05	-82.29	-1.06E-05	-243.14	-1.05E-05	-85.00
S.E	**6.30E-02**	**237.56**	**0.063**	**237.50**	**0.063**	**237.50**

LG23, Legon (2023); LG24, Legon (2024); KW24, Kwadaso (2024); YLD_F, GCA yield of the female inbred line; YLD_M, GCA yield of the male inbred line; S.E, standard error.

Table 5. 7: General combining abilities of the 24 inbreds across optimal environments in Ghana.

	YLD		PH		EH		DYANTH		DYSK	
INBRED	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**	**GCA_F**	**DYSK_M**
1368	7.96E-06	16.90	-0.34	0.00	-0.64	-0.21	0.06	0	0.12	0
9006	-1.83E-05	-117.25	-1.52	0.00	-4.66	-0.55	0.68	0	0.49	0
1368-150GY(119)S	8.29E-06	-62.07	-0.76	0.00	-1.71	-0.21	1.15	0	1.09	0
C316-7	-1.30E-05	-67.60	-0.84	0.00	-1.65	-0.10	1.18	0	0.69	0
DHL 142 POP 1	1.15E-05	-75.20	-1.07	0.00	-0.23	-0.06	-0.63	0	-0.22	0
DHL 18 POP 1	9.15E-06	11.68	-0.53	0.00	2.06	0.08	-1.07	0	-0.77	0
DHL 184 POP 2	-1.13E-05	75.84	-0.54	0.00	-0.07	0.00	-1.29	0	-0.95	0
DHL 186 POP 2	8.01E-06	74.01	0.15	0.00	5.31	0.09	-0.65	0	-0.56	0
DHL 46 POP 1	-1.94E-06	54.48	0.15	0.00	3.86	0.25	-0.19	0	0.03	0
DHL 53 POP 4	-1.10E-05	63.82	2.93	0.00	-2.97	0.34	0.08	0	0.11	0
DHL 99 POP 2	1.87E-05	26.57	-0.34	0.00	1.47	0.31	-0.83	0	-0.72	0
EXP 124	-1.25E-05	71.68	0.20	0.00	-4.34	0.45	0.78	0	0.49	0
HP020-4/1(A)/1/1	-4.02E-06	40.37	-0.83	0.00	-2.06	-0.02	1.19	0	0.55	0
HP020-5/1(B)/1/7	-3.32E-06	14.41	-0.16	0.00	2.12	0.01	0.51	0	0.39	0
HP020-5/2(A)/1/7	4.20E-06	-64.20	-0.50	0.00	1.79	0.10	-1.17	0	-0.90	0
HP020-6/2(9)/9/6	-7.95E-06	-28.65	1.86	0.00	-2.66	-0.07	0.94	0	0.45	0
LMI 102	7.43E-06	48.28	-0.06	0.00	2.45	-0.05	0.04	0	0.10	0
LMI 135	2.22E-05	91.44	2.22	0.00	-4.18	0.15	-0.05	0	-0.24	0
LMI 140	7.70E-06	154.16	0.17	0.00	3.96	0.17	-0.49	0	-0.20	0
LMI 141	1.44E-06	107.78	2.74	0.00	0.27	0.03	-0.98	0	-0.58	0
LMI 144	-9.62E-06	-46.90	-0.41	0.00	0.73	-0.03	0.29	0	0.34	0
TZIL 25 (40C) 0.4 EMS	-1.89E-05	-52.29	-0.98	0.00	-0.44	-0.07	0.54	0	0.37	0
TZIL 25 (43A) 0.4 EMS	1.56E-05	-239.32	-0.56	0.00	2.72	-0.59	0.15	0	0.05	0
TZIL 25 (60E) 0.4 EMS	-1.04E-05	-97.95	-0.98	0.00	-1.15	-0.03	-0.22	0	-0.12	0
S.E	**0.06**	**156.09**	**4.03**	**0.00**	**3.99**	**1.25**	**0.69**	**0**	**0.68**	**0**

YLD, grain yield (kg/ha); 100-KW, 100 kernel weight (g); DYANTH; days to anthesis; DYSK, days to silking; EH; ear height; PH, plant height; PASP, plant aspect; GCA-f, general combining ability effects of female inbred parent; GCA-m, general combining ability effects of male inbred parent; SE, standard error.

toward early flowering, an adaptive advantage under stress. Overall, both female and male GCA estimates revealed significant additive variation across environments (Table 5.8). Inbred lines such as DHL 99 POP 2, DHL 186 POP 2, LMI 102, LMI 135 and LMI 140 consistently exhibited favourable GCA estimates for grain yield and associated traits, making them key parental lines for hybrid development targeting broad environmental adaptability in Ghana.

5.4.2 Heterotic Grouping of Inbred Lines Based on GCA of Multiple Traits (HGCAMT)

Heterotic grouping of the 24 inbred lines were performed across individual, combined and pooled environments. The method, which integrates the GCA effects of multiple traits, effectively differentiated the inbred lines into two to three heterotic groups depending on the environment.

Under the drought environment, the inbred lines were separated into two major groups each containing twelve (12) inbred lines each (Figure 5.1). Group I consisted of inbred lines such as DHL 99 POP 2, HP020-5/1(B)/1/7, DHL 186 POP 2, DHL 46 POP 1, HP020-5/2(A)/1/7, LMI 140 and LMI 102, characterized by positive GCA values for grian yield and adaptive traits. Group II included inbred lines such as DHL 53 POP 4, 1368, DHL 18 POP 1, 9006, and TZIL 25 mutant lines/derivatives, which had negative GCA effects and were this clustered as the opposite heterotic group.

Across low nitrogen conditions, the inbred lines were also clustered into two groups. Group I, the largest group containing thirteen (13) inbreds, also consisted of inbred lines such as 9006, C316-7, and HP020-6/2(9)/9/6 while Group II, containing eleven (11) inbreds, included inbreds with positive female GCA for grain yield such as LMI 140, DHL 186 POP 2, LMI 102, and DHL 142 POP 1 (Figure 5.2).

Table 5. 8: General combining abilities of selected traits of the 24 inbreds across all environments in Ghana.

	YLD		PH		EH		DYANTH		DYSK		KW	
INBRED	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**	**GCA_F**	**GCA_M**
1368	-6.04	-21.66	-1.90	1.14	-0.60	-3.07	0.38	0.42	0.37	0.43	-0.16	-0.13
9006	-70.59	-10.08	-6.67	0.61	-7.07	-5.88	1.21	0.42	0.81	0.09	-0.02	0.27
1368-150GY(119)S	23.11	-22.97	-4.65	-0.58	-3.79	-0.80	1.56*	0.71	2.07	0.73	0.11	-0.48
C316-7	-28.51	-43.21	-5.27	-1.48	-4.12	-3.22	1.46*	0.14	1.18	0.46	-0.03	-0.32
DHL 142 POP 1	37.71	-21.60	-3.18	2.96	0.06	-1.80	-1.09	-0.75	-0.75	-0.56	0.02	0.17
DHL 18 POP 1	5.08	31.31	-2.04	-0.51	4.28	1.63	-1.29	-1.25	-1.01	-1.06	-0.17	-0.25
DHL 184 POP 2	14.20	20.79	-3.88	-0.88	-2.17	-0.28	-1.68*	-0.85	-1.58	-0.43	0.18	0.29
DHL 186 POP 2	38.90	36.28	2.40	-0.24	7.11	1.98	-1.32*	-0.80	-1.66*	-1.06	0.20	0.26
DHL 46 POP 1	28.70	36.04	3.43	0.68	7.55	5.47	0.21	0.66	0.22	0.57	0.07	0.17
DHL 53 POP 4	-45.14	7.32	11.40	0.62	-2.86	3.33	0.62	-0.22	0.79	-0.12	0.01	0.78
DHL 99 POP 2	92.15	30.78	-1.53	-0.43	2.22	2.28	-1.25	-0.46	-1.76*	-0.73	0.10	0.70
EXP 124	-44.10	40.82	0.25	-0.67	-3.80	5.16	1.22	0.43	0.67	0.61	0.04	0.45
HP020-4/1(A)/1/1	-0.26	16.44	-4.60	2.34	-4.94	-1.00	1.88	0.97	1.21	0.25	-0.07	0.14
HP020-5/1(B)/1/7	3.33	-12.12	0.05	-0.44	2.60	0.73	1.72	0.70	1.21	0.78	-0.05	-0.61
HP020-5/2(A)/1/7	6.72	-11.61	-1.94	-1.46	2.01	0.26	-1.79	-1.31	-1.85	-1.12	-0.19	-0.94
HP020-6/2(9)/9/6	-41.62	-57.08	5.91	-1.15	-5.78	-2.18	1.27	0.85	1.29	0.72	-0.07	-1.22
LMI 102	53.63	3.34	4.44	3.31	6.43	1.08	-0.40	0.11	0.08	0.22	0.31	0.92
LMI 135	41.59	52.27	6.21	-0.45	-4.45	1.61	-0.47	0.11	-0.89	0.02	-0.03	-0.09
LMI 140	32.04	91.57	2.80	1.71	7.41	3.94	-1.85*	-0.64	-1.73	-0.86	-0.05	-0.06
LMI 141	-17.00	23.32	10.74	-1.19	0.21	0.25	-1.14	-0.17	-0.31	0.25	0.01	0.24
LMI 144	-25.41	-19.02	-0.52	-0.81	2.53	0.77	-0.04	-0.55	0.07	-0.25	-0.14	-0.15
TZIL 25 (40C) 0.4 EMS	-52.68	-12.40	-4.54	-0.86	-3.00	-0.09	0.46	0.56	0.61	0.29	-0.04	0.39
TZIL 25 (43A) 0.4 EMS	-3.69	-94.33	-2.55	-0.28	2.55	-6.86	0.24	0.59	0.43	0.42	0.00	-0.15
TZIL 25 (60E) 0.4 EMS	-42.12	-64.18	-4.35	-1.94	-2.37	-3.28	0.07	0.34	0.52	0.36	-0.04	-0.36
S.E	**84.76**	**87.30**	**6.24**	**3.58**	**3.30**	**2.95**	**0.68**	**0.60**	**0.81**	**0.68**	**0.38**	**0.71**

YLD, grain yield (kg/ha); KW, 100 kernel weight (g); DYANTH; days to anthesis; DYSK, days to silking; EH; ear height; PH, plant height; GCA-f, general combining ability effects of female inbred parent; GCA-m, general combining ability effects of male inbred parent; SE, standard err

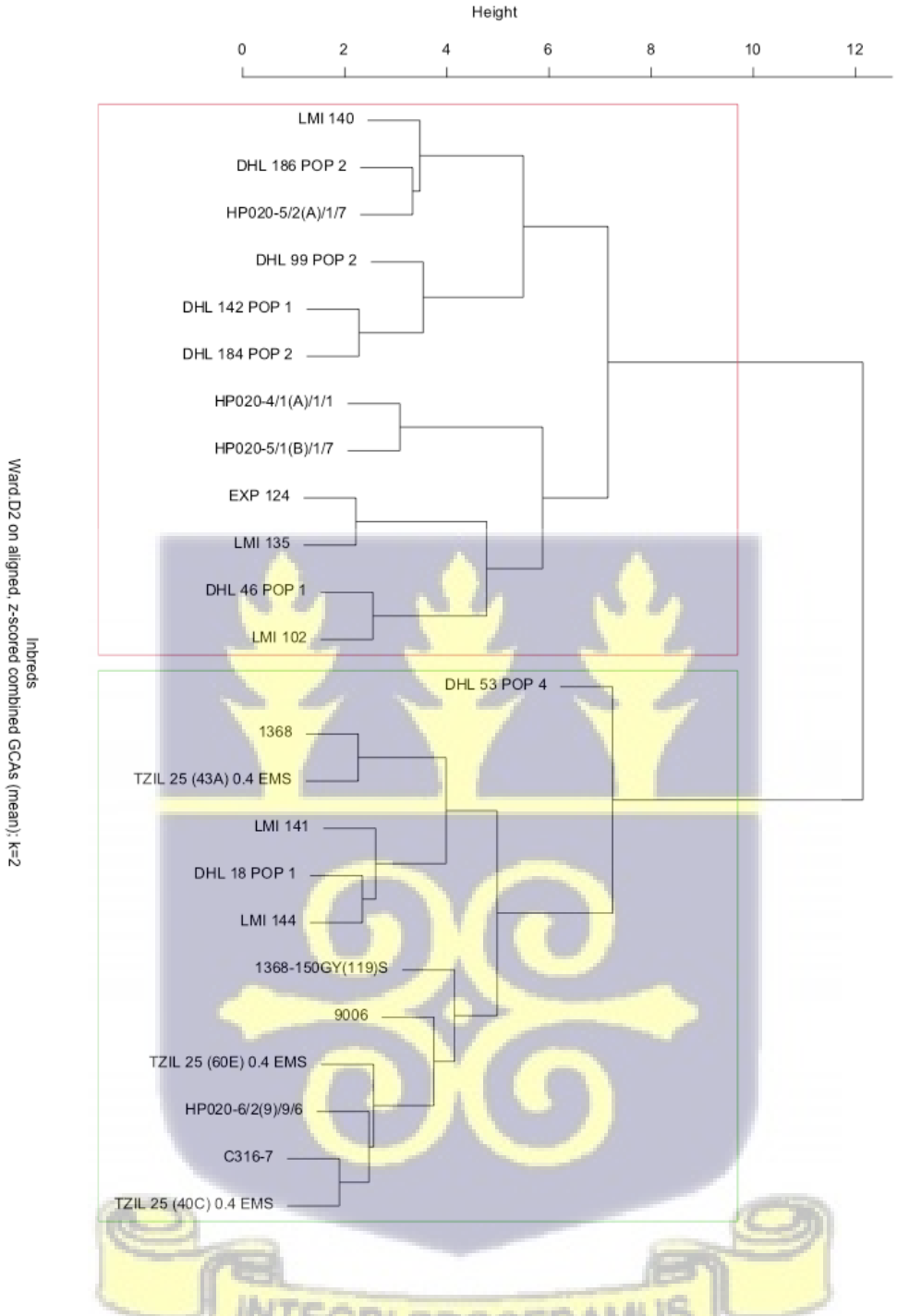

Figure 5. 1: Heterotic grouping on inbred lines under drought environment using the HGCAMT-Ward.D2.

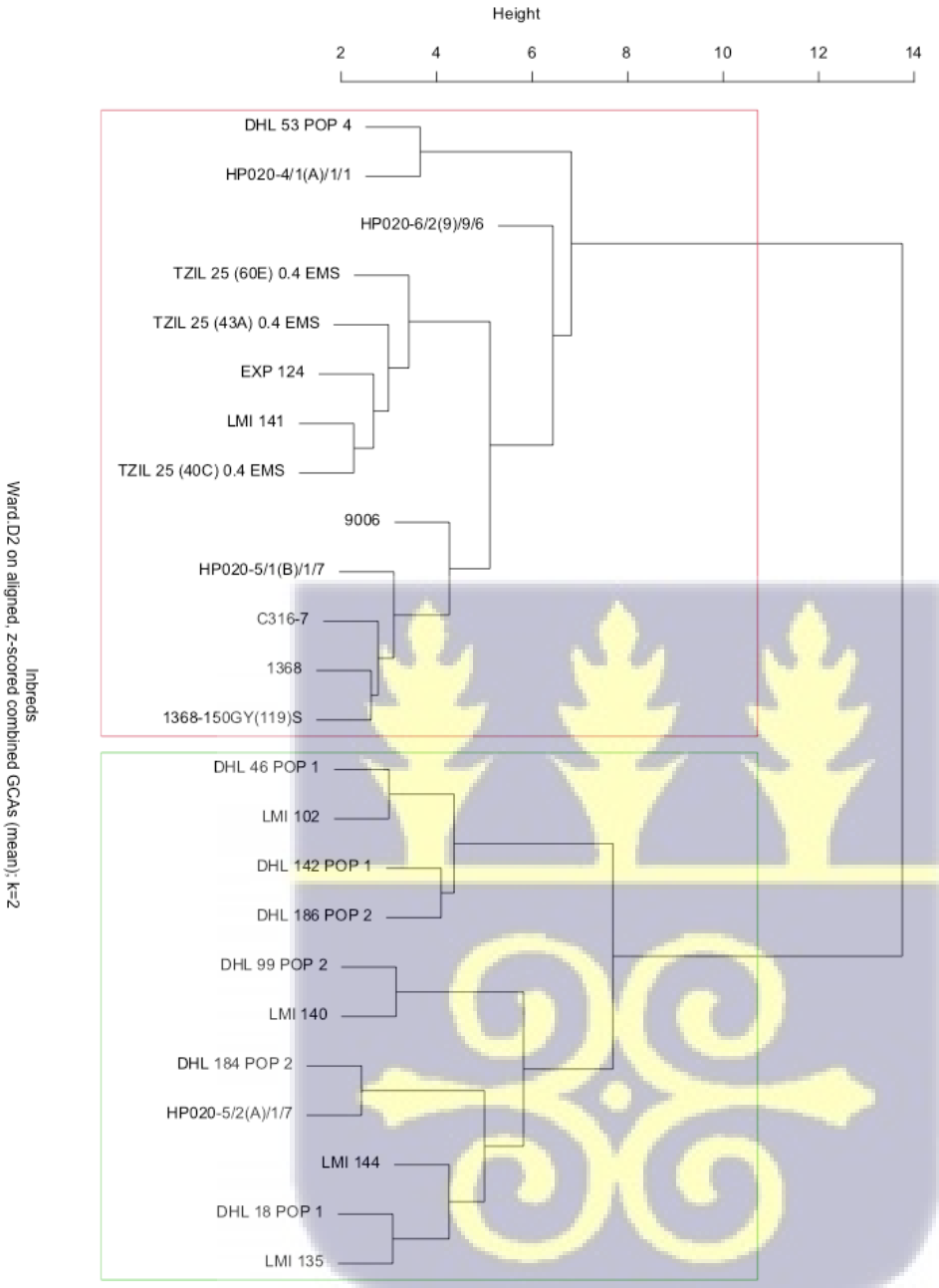

Figure 5. 2: Heterotic grouping on inbred lines across LN environments using the HGCAMT-Ward.D2.

In the combined drought and low-N environment, most of the inbred lines that exhibited positive GCA effects under individual stress environments, including DHL 142 POP 1, DHL 184 POP 2, DHL 99 POP 2 and LMI 135 were clustered together in Group I which contained ten (10) inbred lines (Figure 5.3). Inbred lines such as C316-7, 9006, DHL 53 POP 4 and 1368 were clustered in Group II which was the largest cluster containing fourteen (14) inbred lines.

Across optimal environments, two distinct heterotic groups were formed. Group I, the smallest cluster, contained five (5) inbred lines namely DHL 53 POP 4, EXP 124, HP020-6/2(9)/9/6, LMI 135 and LM1 141 while Group II, the largest cluster, contained nineteen (19) inbred lines including inbreds such as LMI 140, DHL 186 POP 2, DHL 99 POP 2, DHL 184 POP 2 and LMI 102 (Figure 5.4).

Across all environments, the inbred lines were distinctly separated into three heterotic groups (Figure 5.5). Group I, the largest cluster, contained eleven (11) inbred lines such as 1368, LMI 144, 9006, 1368-150GY (119)S and C316-7. Group II contained six (6) inbred lines namely LMI 102, DHL 46 POP 1, EXP 124, DHL 53 POP 4, LMI 135 and LM1 141. Group III contained seven (7) inbred lines namely DHL 99 POP 2, DHL 184 POP 2, DHL 186 POP 2, DHL 142 POP 1, LMI 140, DHL 18 POP 1 and HP020-5/2(A)/1/7.

Overall, the HGAMT-based grouping successfully classified the inbred lines according to their additive genetic performance across environments. The consistent clustering of inbred lines such as LMI 140, DHL 186 POP 2 and DHL 99 POP 2 into the same heterotic group across environments highlights their stability as potential testers, while 9006, C316-7 and TZIL 25 mutant lines consistently being grouped in a different heterotic group (Table 5.9).

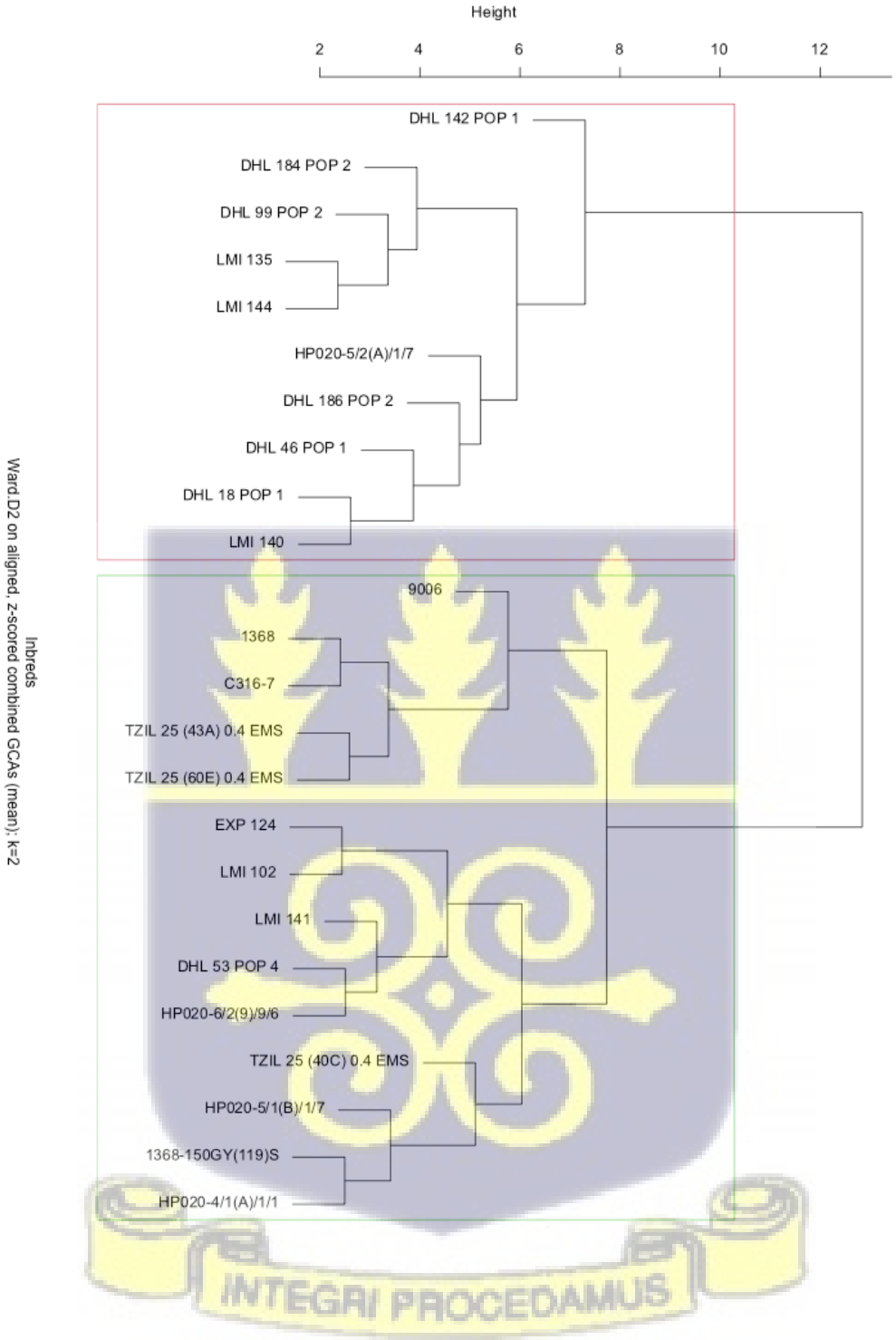

Figure 5. 3: Heterotic grouping of inbred lines under DTLN environment using the HGCAMT-Ward.D2.

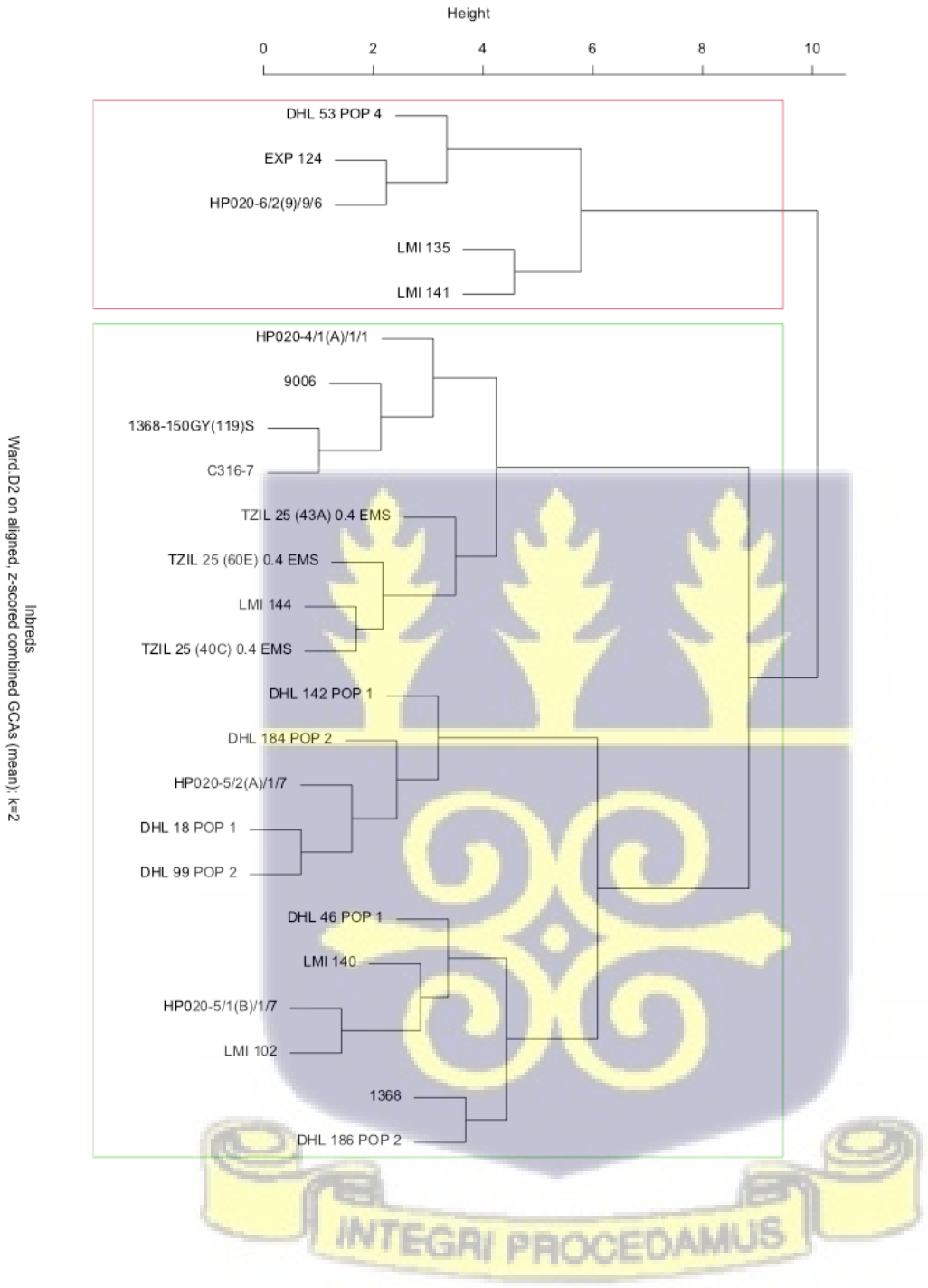

Figure 5. 4: Heterotic grouping of inbred lines across optimal environments using the HGCAMT-Ward.D2.

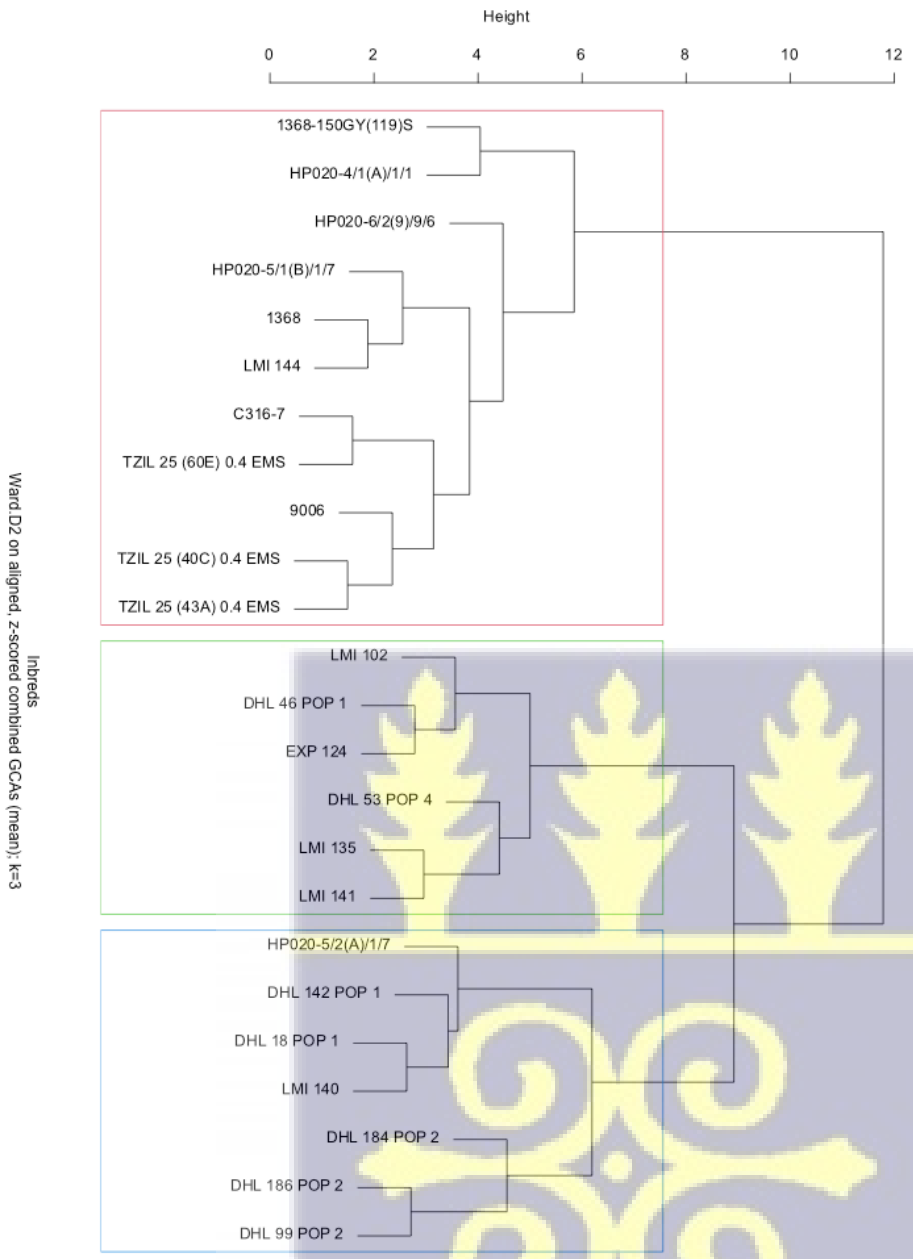

Figure 5. 5: Heterotic grouping of inbred lines across all environments using HGCAMT-Ward.D2.

Table 5. 9: Summary of heterotic groups of the twenty-four maize inbred lines based on the HGCAMT under individual, combined and across contrasting environments.

Method	GROUP 1	GROUP 2	GROUP 3
HGCAMT-drought environment	DHL 99 POP 2, HP020-5/1(B)/1/7, DHL 186 POP 2, DHL 46 POP 1, HP020-5/2(A)/1/7, LMI 140, LMI 102, HP020-4/1(A)/1/1, DHL 142 POP 1, DHL 184 POP 2, EXP 124, LMI 135	DHL 53 POP 4, 1368, 1368-150GY(119)S, DHL 18 POP 1, HP020-6/2(9)/9/6, TZIL 25 (40C) 0.4 EMS, 9006, LMI 144, , TZIL 25 (43A) 0.4 EMS, TZIL 25 (60E) 0.4 EMS, C316-7, LMI 141	
HGCAMT-low soil N environment	9006, TZIL 25 (43A) 0.4 EMS, C316-7, TZIL 25 (60E) 0.4 EMS, HP020-4/1(A)/1/1, HP020-6/2(9)/9/6, 1368-150GY(119)S, 1368,HP020-5/1(B)/1/7, DHL 53 POP 4, EXP 124, LMI 141, TZIL 25 (40C) 0.4 EMS	DHL 99 POP 2, LMI 140, LMI 135, LMI 144, DHL 18 POP 1, HP020-5/2(A)/1/7, DHL 142 POP 1, DHL 186 POP 2, LMI 102, DHL 184 POP 2, DHL 46 POP 1	
HGCAMT-combined drought and low soil N environment	DHL 142 POP 1, DHL 184 POP 2, DHL 99 POP 2, LMI 135, LMI 144, HP020-5/2(A)/1/7, DHL 186 POP 2, DHL 46 POP 1, DHL 18 POP 1, LMI 140,	HP020-6/2(9)/9/6, 9006, 1368, 1368-150GY(119)S, EXP 124, TZIL 25 (43A) 0.4 EMS, LMI 102, TZIL 25 (40)C 0.4 EMS, HP020-5/1(B)/1/7, C316-7, DHL 53 POP 4, HP020-4/1(A)/1/1, LMI 141	,
HGCAMT-optimum environment	DHL 53 POP 4, EXP 124, HP020-6/2(9)/9/6, LMI 135, LMI 141	1368, DHL 18 POP 1, HP020-5/1(B)/1/7, DHL 46 POP 1, LMI 102, DHL 99 POP 2, HP020-4/1(A)/1/1, LMI 140, DHL 184 POP 2, DHL 186 POP 2, TZIL 25 (43A) 0.4 EMS, 9006, TZIL 25 (60E) 0.4 EMS, DHL 142 POP 1, C316-7, 1368-150GY(119)S, HP020-5/2(A)/1/7, , LMI 144, TZIL 25 (40C) 0.4 EMS	
HGCAMT-across environment	9006, C316-7, TZIL 25 (60E) 0.4 EMS, HP020-4/1(A)/1/1, 1368-150GY(119)S, HP020-6/2(9)/9/6, TZIL 25(40C) 0.4 EMS, HP020-5/1(B)/1/7, LMI 144, TZIL 25 (43A) 0.4 EMS, 1368,	LMI 102, DHL 46 POP 1, EXP 124, DHL 53 POP 4, LMI 135, LMI 141	HP020-5/2(A)/1/7, DHL 18 POP 1, DHL 99 POP 2, DHL 184 POP 2, DHL 142 POP 1, DHL 186 POP 2, LMI 140

5.4.3 Relative contributions of combining ability effects

Under the single stress environment (DT and LN), combined environment (DTLN) and across all environments, the relative contribution of GCA (male and female) effects were greater than the SCA effects for grain yield and all measured traits.

The combined GCA effects (GCA-female and GCA-male) under drought varied from 42% for EPP to 80% for days to 50% anthesis (DYANTH) while the SCA effects varied from 20% for DYANTH to 58% for number of ears per plant (EPP) (Figure 5.7). Comparatively, the GCA-female effects for almost all the traits with the exception of EPP. Traits such as ear aspect (EASP) and EPP had higher SCA effects of 55% and 58% respectively. The combined GCA effects accounted for 54% of the total variation for grain yield (YLD) while SCA effects accounted for 47%.

Across low nitrogen environments, the combined GCA effects ranged from 47% for EPP to 76% for DYANTH while the SCA effects ranged from 24% for DYANTH to 44% for YLD (Figure 5.8). The GCA-female effects were higher for all the traits with the exception of stay-green (STGR). With the exception of 100-seed kernel weight (100-KW), leaf senescence (LS) and EPP, the combined GCA effects was higher for all other traits.

The combined GCA effects under the combined (DTLN) environment varied from 44% for STGR to 85% for ear height (EH) (Figure 5.9). The GCA-female effects contributed higher to EH (58%), PH (52%) and DYANTH (50%). For YLD, the combined GCA effects accounted for 52% of the total variation observed.

Across optimum environments, the combined GCA effects ranged from 44% for chlorophyll content (SPAD) to 67% for DYANTH (Figure 5.10). The GCA-female effects were higher for

traits such as DYANTH (50%) and DYSK (42%). With the exception of YLD, ASI, SPAD and EPP, the combined GCA effects was higher for all other traits.

Across all environments, the combined GCA effects ranged from 42% for EPP to 81% for DYANTH (Figure 5.11). Both GCA and SCA effects contributed equally to YLD and STGR. The GCA-female effects contributed higher to traits such as DYANTH (55%) and EH (50%). 100-KW had the highest GCA-male effects of 30%. SCA effects dominated in traits such as PASP (64%), EASP (52%) and EPP (58%).

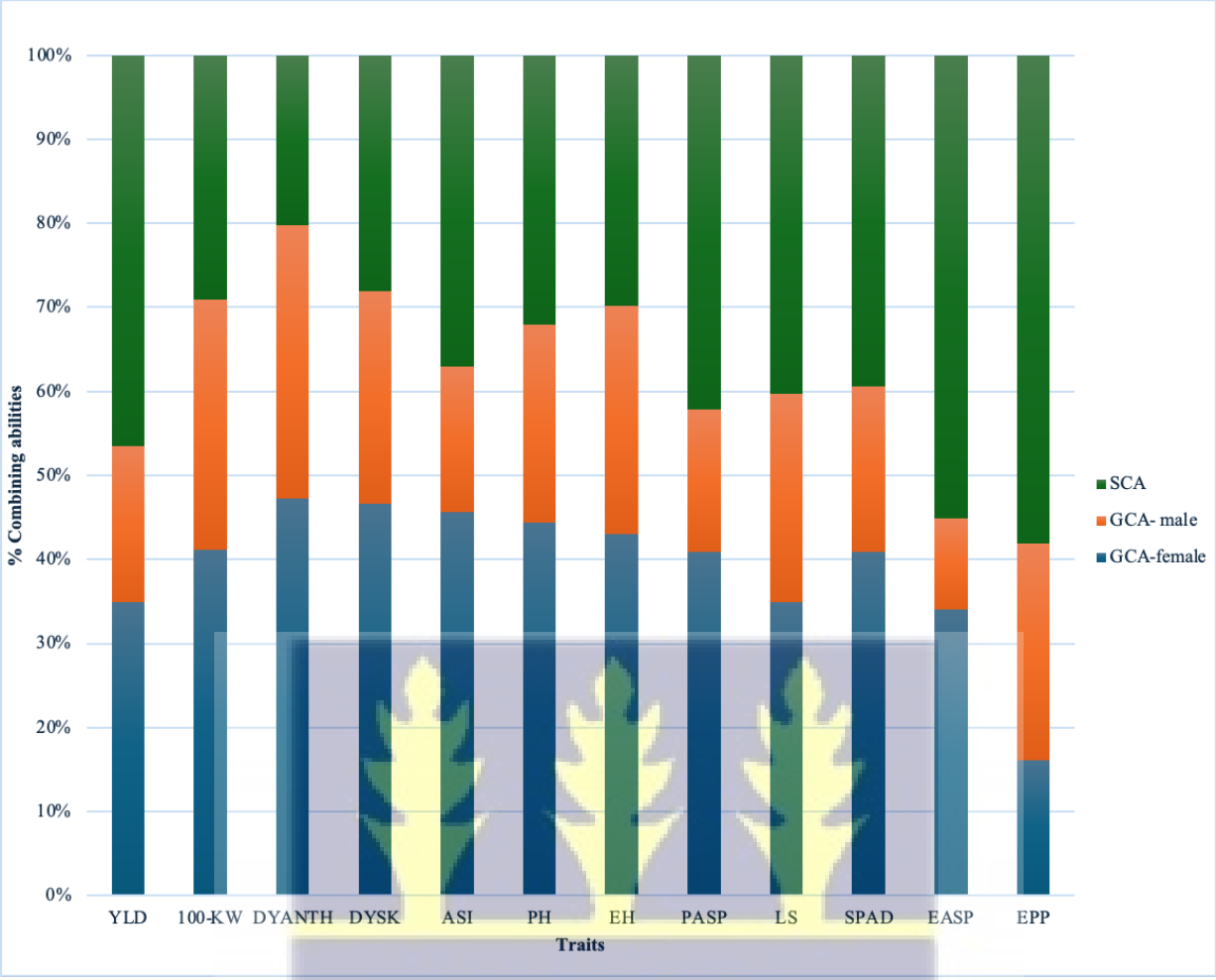

Figure 5. 6: The contribution of general and specific combining abilities to the variability for grain yield and other agronomic traits in the DT environment.

YLD, grain yield (kg/ha); 100-KW, 100-grain kernel weight (g); DYANTH, days to 50% anthesis; DYSK, days to 50% silking; ASI, anthesis-silking interval; PH, plant height (cm); EH, ear height (cm); PASP, Plant aspect; LS, leaf senescence; SPAD, leaf chlorophyll content; EASP, ear aspect; EPP, number of ears per plant.

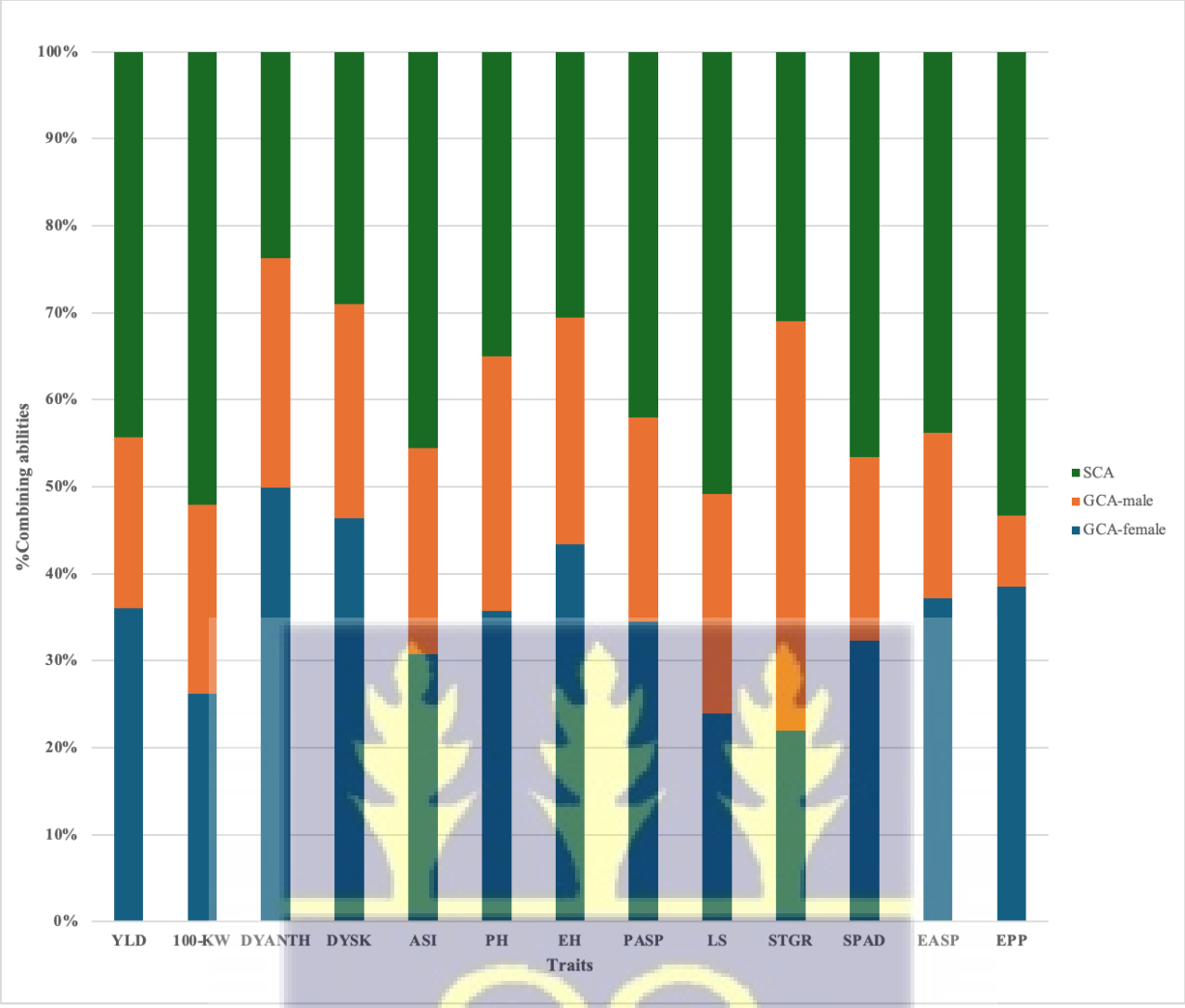

Figure 5. 7: The contributions of general and specific combining abilities to the variability for grain yield and other agronomic traits in the LN environment.

YLD, grain yield (kg/ha); 100-KW, 100-grain kernel weight (g); DYANTH, days to 50% anthesis; DYSK, days to 50% silking; ASI, anthesis-silking interval; PH, plant height (cm); EH, ear height (cm); PASP, Plant aspect; LS, leaf senescence; STGR, stay-green characteristic; SPAD, leaf chlorophyll content; EASP, ear aspect; EPP, number of ears per plant.

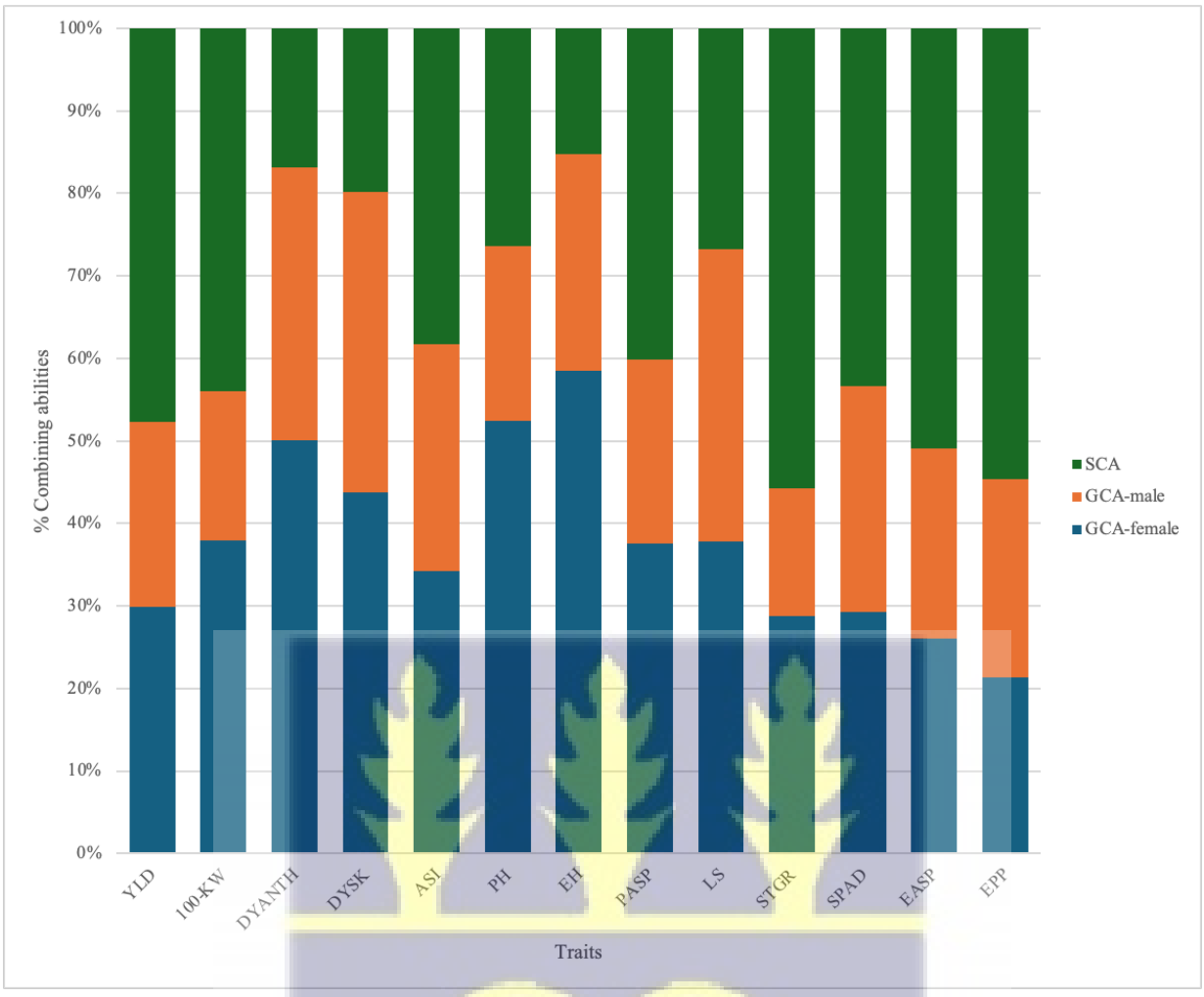

Figure 5. 8: The contributions of general and specific combining abilities to the variability for grain yield and other agronomic traits in the DTLN environment.

YLD, grain yield (kg/ha); 100-KW, 100-grain kernel weight (g); DYANTH, days to 50% anthesis; DYSK, days to 50% silking; ASI, anthesis-silking interval; PH, plant height (cm); EH, ear height (cm); PASP, Plant aspect; LS, leaf senescence; STGR, stay-green characteristic; SPAD, leaf chlorophyll content; EASP, ear aspect; EPP, number of ears per plant.

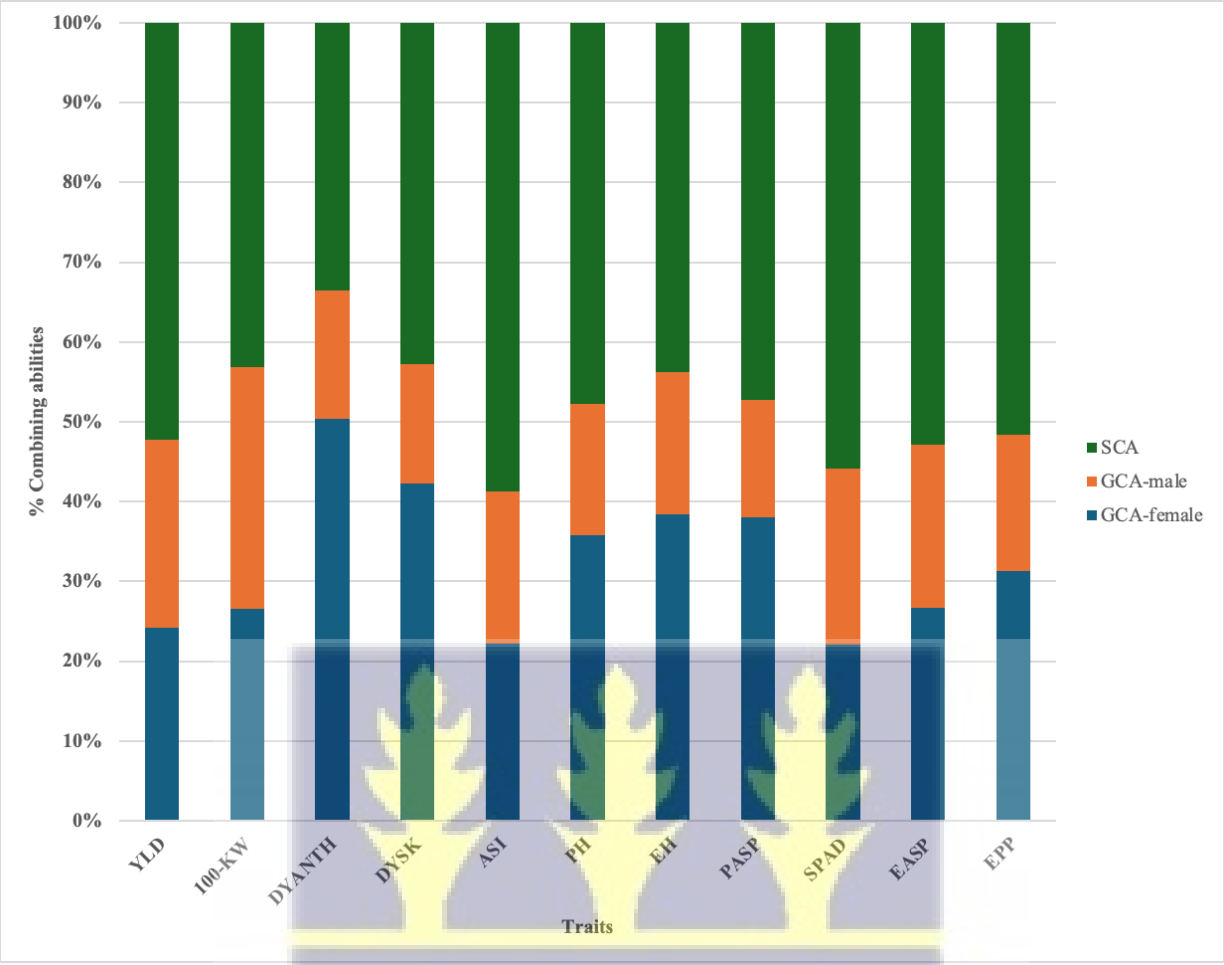

Figure 5. 9: The contributions of general and specific combining abilities to the variability for grain yield and other agronomic traits in the OPT environments.

YLD, grain yield (kg/ha); 100-KW, 100-grain kernel weight (g); DYANTH, days to 50% anthesis; DYSK, days to 50% silking; ASI, anthesis-silking interval; PH, plant height (cm); EH, ear height (cm); PASP, Plant aspect; SPAD, leaf chlorophyll content; EASP, ear aspect; EPP, number of ears per plant.

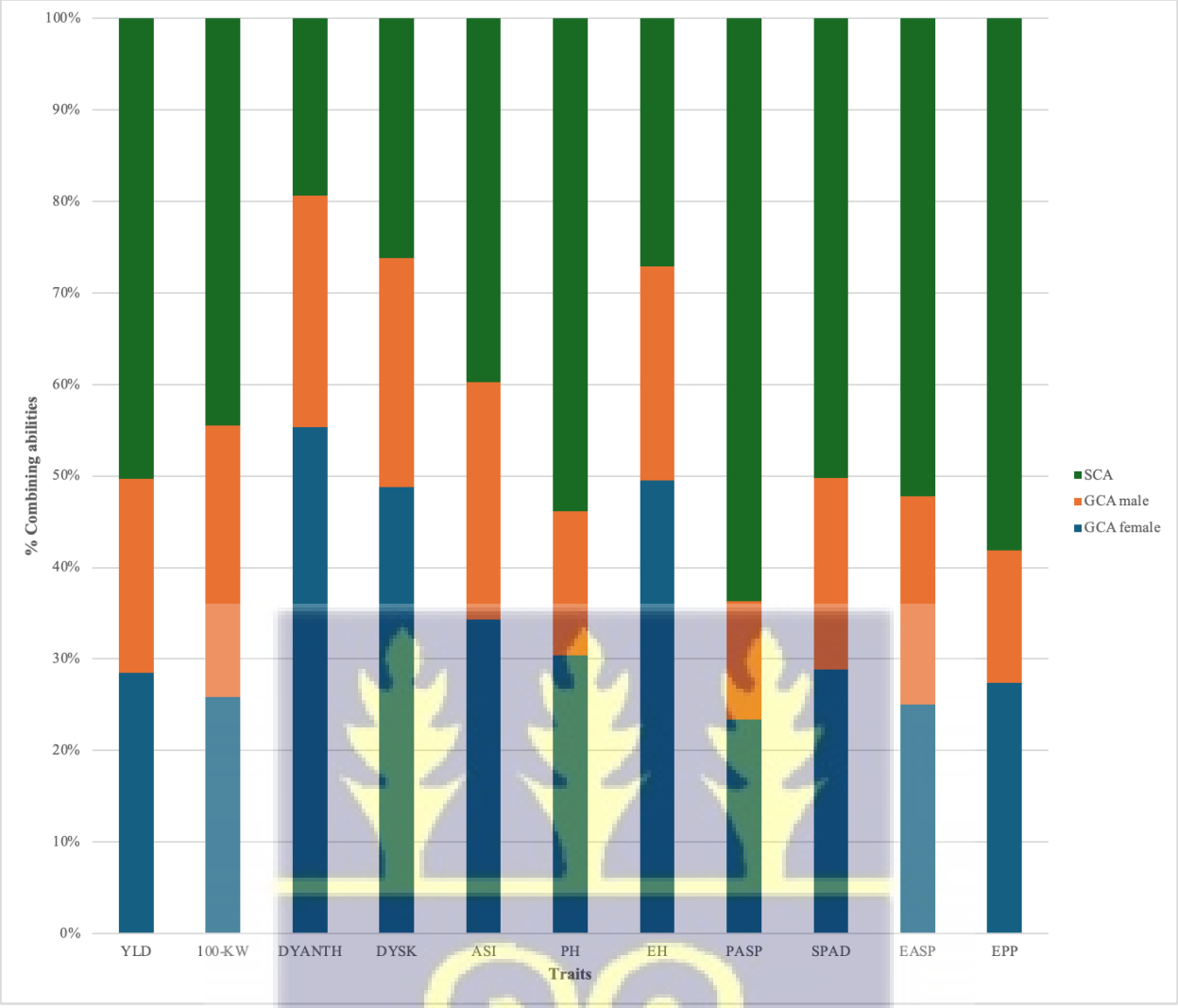

Figure 5. 10: The contributions of general and specific combining abilities to the variability for grain yield and other agronomic traits across all environments.

YLD, grain yield (kg/ha); 100-KW, 100-grain kernel weight (g); DYANTH, days to 50% anthesis; DYSK, days to 50% silking; ASI, anthesis-silking interval; PH, plant height (cm); EH, ear height (cm); PASP, Plant aspect; SPAD, leaf chlorophyll content; EASP, ear aspect; EPP, number of ears per plant.

5.5 Discussion

Under and across the four management conditions, that is drought, low soil nitrogen, combined drought and low nitrogen and optimal environments, the significant variability in GCA effects observed traits indicated that additive gene action largely influenced the inheritance of grain yield and other agronomic traits among the studied maize inbred lines. Under the varying environments, the positive GCA effects recorded for inbred lines such as DHL 99 POP 2, DHL 184 POP 2, DHL 186 POP 2, DHL 142 POP 1 and LMI 102 suggest the presence of favourable additive alleles contributing to improved yield performance. Such inbred lines can be considered good general combiners for stress tolerance and yield potential, indicating that selection based on additive effects could be effective in these lines (Amegbor *et al.*, 2017; Obeng-Bio *et al.*, 2019; Badu-Apraku *et al.*, 2020; Engida *et al.*, 2024).

The low to zero GCA estimates observed for grain yield under the varying conditions are most likely due to singular variance fits arising from data imbalance and minimal additive variation under severe stress, in particular. In the stress environments, many hybrids exhibited low yields, leading to an absence of measurable genetic differentiation among parental lines. This lack of variability reduces the model's ability to partition variance effectively and often results in boundary estimates, where the additive variance component approaches zero. This is corraborated by Port *et al.* (2024) who argued that experimental unbalance can inflate variance comonents, which diminishes the reliability of estimates, but when appropriately managed can allow for meaningful selection gains.

Also, the occurrence of missing or failed hybrid combinations under stress further restricted information available for estimating male effects within the NCII design structure. Consequently, the across LN environment variance for the male main effect of grain yield was estimated zero,

whereas the environment x male interaction variance remianed non-zero. This indicates that male GCA effects for yield were largely environment-specific rather than consistent across environments. Research by Mukaro *et al.* (2023a) indicates that significant interactions for grain yield exist, particularly under varying environmental conditions, which challenges the consistence of male GCA effects across different environments. They emphasize that interactions influencing hybrid perfromance can be affected by environmental stress, complicating the reliable general application of GCA under such conditions. The apparent zero GCA estimates, therefore, should be inteprated as statistical artifacts reflecting data limitations and environmental uniformity, not as evidence of a bioloical absence of additive male contribution (Derera *et al.*, 2008; Osuman *et al.*, 2022d).

The observed variability in GCA effects for plant height and flowering traits further highlights the diverse genetic backgrounds of the inbred lines studied. Negative GCA vlaues for days to anthesis and silking in inbreds such as HP020-5/2(A)/1/7, LMI 140 and DHL 142 POP 1 indicates earliness, a critical adaptive trait under stress conditions (Akaogu *et al.*, 2017b; Osuman *et al.*, 2022d; Sayed *et al.*, 2022; L. M. Yadesa *et al.*, 2021b). Conversly, inbreds such as DHL 46 POP 1 and HP020-4/1(A)/1/1 with positive flowering GCA values express late maturity, which may be beneficial in environments with prolonged growing seasons. The small but consistent positive GCA values for cholorophyll content and delayed leaf senescnece in inbred lines such as DHL 142 POP 1 and HP020-4/1(A)/1/1 indicates a stay-green phenotype, reflecting prolonged photosynthetic activity under stress (Adewale *et al.*, 2023; Nasser *et al.*, 2020b).

While inbred lines with positive GCA effects for traits such as grain yield are likely to transmit their characteristics to their progeny, the lack of statistical significance may indicate variability or environmental influences that obscure the true genetic potential of the inbred line (Badu-Apraku

et al., 2015). In such situations, breeders can rely on the specific combining ability (SCA) estimates to select the best performing hybrids for further evaluation (Badu-Apraku, Fakorede, Gedil, *et al.*, 2016c). SCA is particularly relevant when assessing perfromance in hybrid combinations, accounting for non-additive gene effects that can be pronounced in stress conditions. Studies indicate that SCA estimates can be more informative than GCA values under certain circumstances, especially when evaluating hybrids in varied environments. reported that significant SCA interactions could harness non-additive genetic effects to enhance grain yield under both drought stress and well-watered conditions, reflecting the ability of SCA to capture unique hybrid performance characteristics not evident through GCA alone.

The classification of the twenty-four (24) maize inbred lines into distinct heterotic groups using the HGCAMT method revealed clear patterns of genetic divergence that are consistent with their additve perfromance across environments. The differentiation of lines into two to three groups depending on environmental conditions confirms that the GCA-based grouping approach effectively captured the underlying genetic variability and gene action influencing trait performance. Karim *et al.* (2023) emphasized the importance of using GCA in understanding the genetic architecture of inbred lines, noting that the integration of multi-trait GCA information offers a comprehensive overview compared to single-trait or yield-based grouping methods. This mutlifaceted approach accounts not just for grain yield but also for crucial adaptive traits such as flowering time, plant height and other attributes, ensuring a holistic assessment of the gentic potential of the inbred lines (A. Karim *et al.*, 2023).

As observed, there was moderate similarity in the clustering of inbred lines under and across the contrasting environments. The performance of hybrids can be influenced by environmental factors, which may alter the expression of heterosis. Bhadmus *et al.* (2021) noted that the genetic

variability among early white quality protein maize inbreds, evaluated under low nitrogen and combined drought and heat stress environments, was influenced by the environmental conditions, leading to varying clustering patterns based on the management practices applied. Adu *et al.* (2022) reported significant interactions between general combining ability and environmental conditions, indicating that while some inbred lines maintain consistent performance across varying environments, others may exhibit variable responses. These variations reported by the two studies highlights the importance of evaluating inbred lines under multiple conditions to accurately assess their genetic relationships. Crucial to understanding the adaptability of inbred lines, is the consistency of clustering patterns as highlighted by Oyekale *et al.* (2020b) who found that extra-early biofortified maize inbred lines exhibited varying interactions with environmental factors, yet certain inbred lines were consistently grouped together, suggesting stable genetic relationships. This consistency is critical for plant breeders aiming to select inbreds that perform reliably across different growing conditions.

In addition to the understanding of the contribution of additive (GCA) and non-additive (SCA) components to genetic variance, the North Carolina design II (NC-II) allows breeders to maximize genetic complementation by the interaction between male and female parental lines. By understanding the contributions of male and female parental lines, plant breeders can make more informed decisions about parental selection thereby developing superior varieties that are well adapted to specific environments. According to Badu-Apraku, Bankole, Fakorede, Ogbe, *et al.* (2021) , the NC-II design effectively partitions variance into components associated with male and female effects, thereby enabling a more informed selection strategy among breeders. Their study demonstrates that understanding of both male and female GCA effects and SCA interactions contributes significantly to selecting superior parental lines, allowing researchers to leverage the

strengths of both parents to develop hybrids optimized for specific environmental conditions. Moreover, Mukaro *et al.* (2023b) supports that the use of the NC-II design extends beyond mere understanding of genetic variance. It provides breeders with insights into the complementarity of different parental lines, which is essential in making informed decisions about which lines to combine for optimal adaptability and productivity.

In this study, although GCA effects were predominant, moderate SCA effects for yield and other yield components were also observed under certain environments indicating that non-additive gene action also contributed meaningfully to hybrid performance. Under stress and across all environments, traits such as number of ears per plant, ear aspect and plant aspect recorded relatively higher SCA contributions, implying that hybrid performance for these traits depends on specific parental combinations rather than average parental performance. This agrees with research by Mukaro *et al.* (2023b) which supports the idea that the interplay between GCA and SCA can signifcantly influence hybrid outcomes depending on the environmental conditions. They noted that while GCA is essential for predicting hybrid perfromance, SCA plays a critical role in hybrid performance, especially under varying stress conditions, emphasizing the importance of selecting approporiate parental combinations. This aligns with the findings of Badu-Apraku, Bankole, Fakorede, Ayinde, *et al.* (2021), who also observed notable SCA contributions for certain traits, suggesting a reliance on specific parental combinations for optimal hybrid performance.

The predominance of GCA-female effects for most of the agronomic traits under and across all environments suggests strong maternal additive control, reflecting stable contributions to yield performance and plant adaptation. This phenomenon aligns with Obeng-Bio *et al.* (2019b) whose study highlights that inbred lines exhibiting strong GCA-female effects often yield reliably and adapt favourably to varying environmental conditions. Additionally, Oyekale *et al.* (2020a)

supported the notion that maternal effects influence yield, especially under stress conditions such as Striga infestation and low soil nitrogen. Their findings suggest that a careful selection of female parents could exploit favourable cytoplasmic factors, reinforcing the role of GCA-female effects in enhancing grain yield under different environments.

Overall, the predominance of additive genetic effects across environments demonstrates that recurrent selection and pedigree breeding strategies would be effective for genetic improvement of yield and adaptive traits in the studied germplasm. However, the presence of SCA effects for yield components and the clear heterotic grouping among the lines underscore the need to complement additive selection with heterosis breeding.

5.6 Conclusion

Across drought, low nitrogen, combined drought and low-nitrogen, and optimal conditions, significant variation in combining baility was detected among the twenty-four inbred lines. Several inbred lines, that is DHL 99 POP 2, DHL 186 POP 2, LMI 102, LMI 135 and LMI 140, exhibited favourable GCA effects for grain yield and other key morphological traits, identifying them as reliable general combiners. SCA effects were present but trait-dependent, inidcating opportunities to exploit specific parental combinations for such traits. Apparent near-zero or boundary GCA estimates observed in certain stress conditions reflect data sparsity or imbalance and reduced phenotypic differentiation under severe stress rather than a bioligical absence of additive effects.

There was preponderance of additive gene action (GCA) over non-additive gene action (SCA) in the inheritance of grain yield and most measured traits under and across all environments. However, non-additive gene action, although less significant, played an essential role for traits such as EPP, EASP and PASP, and for grain yield under favourable conditions. Across environments, female GCA effects exceeded male GCA effects for several traits, consistent with stable maternal additive influence, whereas male GCA for yield was larger but moore environment-specific and sometimes poorly estimated due to missing or failed hybrids. These observed patterns support a breeding strategy that emphasizes recurrent/pedigree selection complemented by heterosis breeding.

The twenty-four inbred lines were grouped into two to three heterotic groups. Inbred lines such as DHL 186 POP 2, DHL 99 POP 2, LMI 102, LMI 140 and LMI 135 clustered together repeatedly across environments, highlighting their stability and suitablilty as testers or parental lines for broad -adaptation breeding. Conversely, 9006, C316-7 and the TZIL 25 mutant derivatives were repeatedly assigned to opposing groups, providing contrasting pools for heterosis maximization.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study examined the gentic diversity, combining ability and performance stability of maize inbred lines and their hybrids under varying environmental conditions, including optimal, drought, low soil nitrogen, combined drought and low nitrogen, and herbicide-treated environments. The findings collectively underscore the presence of substantial genetic variability, exploitable additive and non-additive effects, and environments-dependent expression of adaptive traits that can be harnessed fof genetic improvement in tropical maize breeding programmes.

The predominance of additive gene action (GCA) for yield and key adaptive traits across environments suggests that recurrent and pedigree selection strategies would be effective for improving stress tolerance and yield stability. However, the observed non-additive (SCA) effects for traits such as ears per plant, ear aspect, and plant aspect indicate that heterosis breeding remains valuable for exploiting specific parental combinations, particularly under stress-prone environments.

Under herbicide stress environments, there was significant variability in tolerance and mortaility rates among inbred lines highlighted the genotype-dependent response to nicosulfuron. Inbred lines such as C316-7, DHL 99 POP 2, and and DHL 258 POP 2 exhibited low mortality and high yield potential, demonstrating inherent tolerance mechanisms and suitability for use in herbicide-resilient hybrid development. The timing of herbicide application also influenced plant injury, with applications at the V10-VT growth stage showing reduced phytotoxicity compared to early growth stages, underscoring the importance of growth-stage specific management practices.

Under drought and low nitrogen conditions, several inbred lines, particularly DHL 101 POP 5, EXP 124, TZIL 25 (35G) 0.4 EMS DHL 258 POP 2, and TZIL 25 (60E) 0.4 EMS, displayed high yield stability index and low stress susceptibility index, confirming their potential for stress tolerance. These genotype represent valuable parental materials for developing hybirds resilient to multiple stresses.

Molecular characterization using ISSR markers revealed a high level of polymorphsim, confirming a broad genetic base among the inbred lines. Despite the predominance of a single major cluster, the presence of minor clusters indicated useful genetic divergence for heterotic grouping and heterosis exploitation.

Across hybrid evaluations, significant GCA and SCA effects under multiple environments reaffirmed that both additive and non-addtive gene actions influence the inheritance of grain yield and other agronomic/adaptive traits. The preponderance of additive gene action (GCA) over non-additive gene action (SCA) , particularly under combined drought and low nitrogen environments, supports the application of recurrent selection for cumulative genetic gain, while specific SCA contributions to yield components justify exploiting heterotic potential in selected hybrid combinations. Hybrids such as LMI 102 X LMI 140, 1368 X DHL 186 POP 2, HP020-4/1(A)/1/1 X DHL 46 POP 1 and TZIL 25 (40C) 0.4 EMS X TZIL 25 (60E) 0.4 EMS exhibited superior performance in specific environments whereas DHL 99 POP 2 x 9006 and DHL 99 POP 2 x EXP 124 demonstrated broad adaptability across multiple environments.

The multi-environment GGE biplot analysis identified three distinct mega-environments, providing a framewrok for location-specific hybrid recommendation and targetted breeding. DHL 99 POP 2 x 9006 and 1368-150GY(119)S x DHL 184 POP 2 were the most stable, while LMI 102

X LMI 140 was identified as the highest yielding hybrid across environments but showed moderate stability.

Overall, the consistent performance of certain inbred lines and hybrids across diverse conditions highlights their suitability for inclusion in maize breeding pipleines aimed at developing stress-tolerant and high-yielding varieties to sub-Saharan Africa's variable production systems.

6.2 Recommendations

Based on the conclusions from this study, the following recommendations are made:

- The consistent performance of C316-7, DHL 258 POP 2, DHL 99 POP 2, LMI 102 and TZIL 25 (60E) 0.4 EMS under drought, herbicide-control and optimal environments highlights their genetic resilience, hence should be prioritized in the WACCI maize breeding programme and incorporated into hybridization programmes focusing on stress tolerance.
- DHL 65 POP 3, DHL 66 POP 5, DHL 77 POP 2, DHL 130 POP 3, TZIL 25 (35G) 0.4 EMS, C316-7, DHL 258 POP 2, TZIL 25 (60E) 0.4 EMS, DHL 99 POP 2 and HP020-1/4/3/3 exhibited herbicide tolerance and could be used to develop herbicide-tolerant hybrids.
- Nested Association Mapping (NAM) and Multi-Parent Advanced Generation Inter-Cross (MAGIC) populations should be developed using the identified superior inbred lines to combine complementary stress-tolerant alleles from diverse backgrounds.
- The predominance of additive gene action, recurrent and pedigree selection should be prioritized for cumulative genetic gains in yield and adaptive trait, while non-additive gene

effects observed for certain traits justify the concurrent use of heterosis breeding to exploit specific parental combinations.

- Molecular tools such as genomic selection and marker-assisted breeding should be employed to introgress key genes associated with drought tolerance; nitrogen use efficiency and herbicide resistance into elite lines.
- Hybrids such as LMI 102 X LMI 140, 1368 X DHL 186 POP 2 and DHL 99 POP 2 x 9006, identified as superior or stable, should undergo multi-location testing across diverse agro-ecological zones to confirm stability, adaptability, and commercialization potential.
- HP020-4/1(A)/1/1 X DHL 142 POP 1, and TZIL 25 (40C) 0.4 EMS X TZIL 25 (60E) 0.4 should be further tested in their winning locations and environments. These can be recommended for location- and stress-specific conditions. The hybrids, and HP020-4/1(A)/1/1 X DHL 46 POP should be prioritized for high-yield environments.
- To mitigate estimation artefacts under severe stress, future experiments should prioritize balanced mating sets, improved seed set under stress and replication across sites/seasons and should include marker/genomic information to stabilize variance component estimation and improve parental choice.

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APPENDIX

Appendix 1: Pedigree of the 100 maize inbred lines used in the study

Inbreds	Kernel Colour	Pedigree/Population	Source
DHL 122 POP 1	Y	Early Thai	Senegal
DHL 46 POP 1	Y	Early Thai	Senegal
DHL 86 POP 1	Y	Early Thai	Senegal
DHL 14 POP 1	Y	Early Thai	Senegal
DHL 18 POP 1	Y	Early Thai	Senegal
DHL 35 POP 1	Y	Early Thai	Senegal
DHL 14 POP 1 (A)	Y	Early Thai	Senegal
DHL 21 POP 1	Y	Early Thai	Senegal
DHL 31 POP 1	Y	Early Thai	Senegal
DHL 159 POP 1	Y	Early Thai	Senegal
DHL 142 POP 1	Y	Early Thai	Senegal
DHL 29 POP 1	Y	Early Thai	Senegal
DHL 139 POP 1	Y	Early Thai	Senegal
DHL 119 POP 1	Y	Early Thai	Senegal
DHL 194 POP 1	Y	Early Thai	Senegal
DHL 71 POP 1	Y	Early Thai	Senegal
DHL 145 POP 1	Y	Early Thai	Senegal
DHL 258 POP 2	W	Obatanpa	Senegal
DHL 184 POP 2	W	Obatanpa	Senegal
DHL 99 POP 2	W	Obatanpa	Senegal
DHL 169 POP 2	W	Obatanpa	Senegal
DHL 51 POP 2	W	Obatanpa	Senegal
DHL 89 POP 2	W	Obatanpa	Senegal
DHL 174 POP 2	W	Obatanpa	Senegal
DHL 104 POP 2	W	Obatanpa	Senegal
DHL 223 POP 2	W	Obatanpa	Senegal
DHL 160 POP 2	W	Obatanpa	Senegal
DHL 77 POP 2	W	Obatanpa	Senegal
DHL 186 POP 2	W	Obatanpa	Senegal
DHL 121 POP 2	W	Obatanpa	Senegal
DHL 116 POP 2	W	Obatanpa	Senegal
DHL 224 POP 2	W	Obatanpa	Senegal
DHL 145 POP 2	W	Obatanpa	Senegal
1368-250GY (21)B	W		WACCI
DHL 223 POP 2 (A)	W	Obatanpa	Senegal
1368-150GY(89)	W		WACCI

DHL 166 POP 2	W	Obatanpa	Senegal
DHL 109 POP 2	W	Obatanpa	Senegal
DHL 156 POP 3	Y	Suwan 1	Senegal
DHL 47 POP 3	Y	Suwan 1	Senegal
DHL 65 POP 3	Y	Suwan 1	Senegal
DHL 9 POP 3	Y	Suwan 1	Senegal
DHL 119 POP 3	Y	Suwan 1	Senegal
DHL 130 POP 3	Y	Suwan 1	Senegal
DHL 19 POP 3	Y	Suwan 1	Senegal
DHL 112 POP 3	Y	Suwan 1	Senegal
DHL 81 POP 3	Y	Suwan 1	Senegal
DHL 148 POP 3	Y	Suwan 1	Senegal
DHL 22 POP 3	Y	Suwan 1	Senegal
DHL 109 POP 3	Y	Suwan 1	Senegal
DHL 179 POP 3	Y	Suwan 1	Senegal
DHL 60 POP 3	Y	Suwan 1	Senegal
1368-150GY (119)S	W		WACCI
DHL 53 POP 4	W	87036(A)	IRAD
DHL 45 POP 4	W	87036(A)	IRAD
DHL 71 POP 4	W	87036(A)	IRAD
DHL 44 POP 4	W	87036(A)	IRAD
TZIL 25 (43A) 0.4 EMS	W		WACCI
TZIL 25 (60E) 0.4 EMS	W		WACCI
TZIL 25 (64D) 0.4 EMS	W		WACCI
TZIL 25 (35G) 0.4EMS	W		WACCI
TZIL 25 (40C) 0.4 EMS	W		WACCI
DHL 72 POP 5	W	TZE-W-DT C4 STR	IITA
DHL 101 POP 5	W	TZE-W-DT C4 STR	IITA
DHL 138 POP 5	W	TZE-W-DT C4 STR	IITA
DHL 131 POP 5	W	TZE-W-DT C4 STR	IITA
DHL 142 POP 5	W	TZE-W-DT C4 STR	IITA
DHL 65 POP 5	W	TZE-W-DT C4 STR	IITA
DHL 106 POP 5	W	TZE-W-DT C4 STR	IITA
DHL 148 POP 5	W	TZE-W-DT C4 STR	IITA
DHL 19 POP 5	W	TZE-W-DT C4 STR	IITA
DHL 107 POP 5	W	TZE-W-DT C4 STR	IITA
LMI 135	Y		WACCI
LMI 144	Y		WACCI

LMI 102	Y		WACCI
HP020-2/5(9)/1/6	Y		WACCI
1368	W	Across 7721 BC2 x TZSR	IITA
LMI 141	Y		WACCI
LMI 137	Y		WACCI
HP020-4/1(A)1/1	Y		WACCI
HP020-4/4/1/6	Y		WACCI
HP020-1/4/3/3	Y		WACCI
HP020-5/4/6/2	Y		WACCI
TZIL 25	W		
LMI 140	Y		WACCI
M131	W		IRAD
9006	W		IRAD
HP020-5/1(B)/1/7	Y		WACCI
EXP 124	W		
TZMI 760	W		
HP020-6/2(9)/9/6	Y		WACCI
HP020-1/3(10)/1-12/9	Y		WACCI
HP020-5/3/2/3	Y		WACCI
HP020-4/1(B)/1/1	Y		WACCI
C316-7	W		
CLRCYQ 40	Y		
HP020-6/1(9)/1-7/2	Y		WACCI
HP020-5/2(A)/1/7	Y		WACCI
HP020-4/3/1-5/1	Y		WACCI