

**COMBINING ABILITY AND GENOTYPE BY
ENVIRONMENT INTERACTION OF SELECTED MAIZE
INBRED LINES FOR PERFORMANCE UNDER LOW
NITROGEN AND DROUGHT STRESS**

**NYOMBAYIRE ALPHONSE
B.Sc. (Agric) National University of Rwanda (NUR), Butare**

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DECLARATION

This thesis is my original work and has never been submitted for a degree in any other university

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.....

Nyombayire Alphonse

Date

This thesis has been submitted for examination with our approval as university supervisors

.....

.....

Dr. Edema Richard

Date

(B.Sc (Agric.) (MAK); M.Sc. (MAK); PhD, (Ohio State University)

.....

.....

Dr. Asea Godfrey

Date

(B.Sc (Agric.) (MAK); M.Sc. (MAK); PhD, (Ohio State University).

DEDICATION

To my God, the Father, the Son and the Holy Spirit,

My wife, Goreth Umutesi, my daughter, Ketsia A. Gutabarwa and my son Blessed A. Iratuzi,

My parents, brothers and sister.

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ACRONYMS AND ABBREVIATIONS

ANOVA:	Analysis of Variance
AMMI:	Additive Main Effects and Multiplicative Interaction
ASI:	Anthesis-Silking Interval
CIMMYT:	Centro Internacional del Mejoramiento de Maiz y Trigo
CML:	CIMMYT Mexico line
CZL:	CIMMYT Zimbabwe Line
EPP:	Number of Ears per Plant
GCA:	General Combining Ability
GEIs:	Genotypes by Environments Interactions
GY:	Grain Yield
IPCA:	Interaction Principal Component Axes
KARI:	Kenya Agriculture Research Institute
GGE:	Genotype and Genotype by Environment
Lsene:	Leaf senescence.
NaCRRI:	National Crops Resources and Research Institute
PCA:	Principal Component Analysis
SCA:	Specific Combining Ability
SC:	Single Cross

ABSTRACT

Harsh drought and infertile soils have increasingly become important abiotic stresses affecting maize production in Sub-Saharan Africa. Varieties with improved water use efficiency and/or N would be beneficial for subsistence farmers. Unfortunately, few breeding Programs focus attention on breeding for low N and drought stress conditions. CIMMYT Breeding Programs in Sub-Saharan Africa in the recent past has devoted their effort to breed for low N and drought tolerance and several germplasm including inbred lines and populations have been developed and availed to the National Programs and some of the elite inbred lines have now been acquired by the Maize Program at the National Agricultural Crop Resources Research Institute (NaCRRI) in Uganda. The main objective of this study was to assess performance of selected group of CIMMYT maize inbred lines to achieve superior hybrids with tolerance to drought and low soil nitrogen stress. The specific objectives were to determine: (1) the combining ability (GCA and SCA) of selected maize inbred lines for performance under drought stress and low nitrogen, including the interaction of GCA and SCA with environments, (2) the effects of low-N and drought stress on yield of single crosses of selected inbred lines, (3) the relationship of grain yield with other traits that might be used for indirect selection under stress, and finally (4) the patterns of G x E interactions of hybrids. Twelve parents were crossed in a half-diallel mating design. The resultant F1 hybrids were evaluated from 2008B-2009B in four managed environments (optimum N and low-N, well-watered and managed drought-stress) in two locations, namely, Kenya Agriculture Research Institute (KARI) substation at Kiboko -Kenya and National Crop Resources Research Institute (NaCRRI) Namulonge-Uganda. Data was collected on days to anthesis, days to silking, anthesis–silking interval, ears per plant, leaf senescence and yellowing, and grain yield.

Results obtained indicated significant variation among the single-cross hybrids for grain yield and other agronomic traits. Additive genetic effects for grain yield (GY), number of ears per plant (EPP) and anthesis-silking interval (ASI) were generally more important than were non-additive genetic effects.

The narrow-sense coefficient of genetic determination (NS-CGD, analogous to heritability, but for a fixed set of parents) was low for grain yield in stress environments, but was high for ASI and leaf senescence. Genotype by environment variance contribution to the total phenotypic variance was much higher than was the genotypic contribution across stress environments (46% vs 3%). The average grain-yield reduction due to stress was 76 % under drought and 50 % under low-N environments. Strong correlations were observed between yield and ASI (-0.67) and EPP (0.80) under drought stress. Some of the traits (ASI, leaf senescence) also exhibited high NS-CGD and, therefore, confirming that they can be used for indirect selection of performance under drought and low-N stress. AMMI and GGE showed that hybrid G8 (CZL 00003 x CML 341) was most high yielding and stable variety. Inbred lines CZL 02004 and CZL 03002 consistently exhibited positive GCA effects for grain yield in all environments. The most beneficial GCA effects for ASI were shown by inbred lines CZL 02004 under managed drought stress and by CML 379 under low-N. Inbred lines with good potential for hybrid production include CZL 02004, CZL 03002, CML 78, and CML 379. These inbred lines had the highest positive GCA effects for grain yield and most beneficial GCA for ASI across stress conditions tested and appear to be suitable for the low soil fertility and drought prone environments. Selection for performance in drought and low-N stresses should focus on high yielding hybrids from parents with desirable GCA values for yield, ASI, and EPP under drought stress.

CHAPTER ONE

INTRODUCTION

1.1 Background and rationale of the study

Globally, maize is ranked second after wheat among cereals, with about 600 million ton produced annually (Meng and Ekboir, 2001). Close to 700 million tons of maize grain was produced in 2004 making it the biggest crop grown worldwide (FAO, 2004). Of these about 28 million tons were produced in sub-Saharan Africa. That year eastern Africa alone produced 6 million tons.

In sub-Saharan Africa, maize is considered a very important food and feed crop that provides protein and most of the energy source for humans and livestock (Bänziger and Diallo, 2001; Diallo *et al.*, 2001; Ngaboyisonga, 2008). More than 300 million Africans depend on maize as their staple food (Bänziger and Diallo, 2001) where it serves both rural and urban populations. In addition to the high demand as food in Africa, maize is also fast becoming a very important agricultural export crop within the region (Asea, 2005). An even higher demand is projected with the region's rising population growth and expanding need for livestock feed. Therefore, it is crucial to increase maize production as a crop essential to food security.

In many developing countries, such as, Malawi, Kenya, Zambia and Zimbabwe, maize is the basic staple food with a per capita consumption average of over 100 kilograms per year that supplies 40% of the total calorie needs (Bänziger and Diallo, 2001; Morris,

1998). The demand for maize in developing countries is expected to surpass the demand for both wheat and rice by the year 2020 (Pingali and Pandey, 2001). From 1995 to 2020, global and sub-Saharan Africa consumption is projected to increase by 50% and 93% respectively.

However, maize suffers from a wide range of production constraints, the most important being the increasingly drought incidence and infertile soils (Meseke, 2008; Edmeades *et al.*, 2006). In the tropics, drought and low soil fertility (mainly nitrogen deficiency), frequently occur together. Drought is one of the important constraints to crop production. It even affects crops grown in subtropical and mid altitude environments when rainfall is erratic (Diallo *et al.*, 2001). Similarly, nitrogen is the most limiting nutrient in the lowland and mid-altitude agro ecologies in the tropics and worst still a considerable proportion of maize in these areas is grown by small scale farmer under low nitrogen conditions (Bänziger *et al.* 2004). Drought affects agricultural production on about 60% of the land area in the tropics and destroy entire maize yields over large expanses annually (Pingali, 2001). This amounts to an estimated 16 million tons of grain loss (Edmeades *et al.*, 1992; Bänziger and Diallo, 2001)

Supplementary irrigation could potentially improve maize production in drought prone areas (Boyer and Westgate, 2004; Derera *et al.*, 2008; FAO, 2006). However, the majority of smallholder farmers cannot access irrigation either because of their geographical location or high capital cost to develop irrigation infrastructure or reliable

water source. Only about 5% of the cropped area in developing countries is irrigated (FAO, 2006; Bänziger and Diallo, 2001).

Nitrogen deficiency is almost universal in the tropics except on recently cleared land (Bellon, 2001), and is one of the most important abiotic factors limiting maize yields (Lafitte and Bänziger, 1997, Bänziger and Diallo, 2001; Diallo *et al.*, 2001;). This means that the nitrogen requirement of the crop must be met by the addition of organic or inorganic fertilizers. However the high cost of inorganic fertilizers makes it unaffordable to the majority of the subsistence farmers in most African countries. In these countries, fertilizer consumption on crop land averages 25kg/ha and seems to have decreased over the period from 1993 to 2003 (FAOSTAT, 2003) as farmers have faced increased input costs and decreasing production price (Bänziger *et al.*, 2004).

Currently, there are few maize varieties that tolerate drought and/or low nitrogen. Few of these varieties are early maturing simply because commercial breeders target commercial farmers who prefer high yielding intermediate to late maturing varieties. Intermediate to late maturing maize varieties are preferred for commercial production because of their high yield potential. Maize in the tropics continues to be exposed to different forms of drought and nitrogen stress for three main reasons: (1) the global climatic changes, (2) the displacement of maize to more difficult production environments by high value crops, and (3) the declining soil organic matter reducing soil fertility and water holding capacity (Bänzinger and Cooper, 2001).

Efforts to improve maize productivity have focused on producing high yielding and high input varieties (Bänziger and Lafitte, 1997; Bänzinger and Cooper, 2001). Further efforts have been directed at improving crop management and soil fertility through several organic and inorganic amendment options. Unfortunately, all these efforts will largely benefit the average small-holder and commercial farmers but not the resource poor farmers. A better approach to help these poor subsistence farmers is by using varieties that utilize nitrogen more efficiently as well as tolerating the periodic droughts which befall the region. Another way is to develop varieties that either escape or tolerate the stress

The International Maize and Wheat Improvement Center (CIMMYT), through conventional breeding, has made significant progress in developing maize germplasm tolerant to drought and low nitrogen (Bänziger *et al.* 2000; Bänzinger and Cooper, 2001; Edmeades *et al.*, 2006). Several germplasm that includes inbred lines and populations have been developed through different breeding programs within CIMMYT. Some of this germplasm has now been acquired by the Maize Program at the National Agricultural Crop Resources Research Institute (NaCRRI) in Uganda.

The germplasm available as inbred lines can be used to develop hybrids and synthetics. However, determination of general combining ability (GCA) and specific combining ability (SCA) of these maize inbred lines to drought and low soil nitrogen stress is important to design appropriate breeding strategies. Knowledge of the combining ability of this germplasm would be very beneficial to the NaCRRI breeders in deciding how to

develop best single-cross hybrids, three-way cross hybrids, or synthetic varieties from these lines.

Furthermore, the performance of hybrids generated across different agro-ecologies/environments also needs to be assessed. Because maize genotypes differ in their adaptation to environments, understanding the magnitude of genotype by environment (G x E) interaction would lead to identification of specific adaptations and stable hybrids across environment. Finally, selection for drought tolerance is still a daunting task (Bänzinger *et al.*, 2004; Bänzinger *et al.*, 1997; Bänzinger and Cooper, 2001). An indirect selection of performance under stress has been proposed (Richards, 2006; Bolaños and Edmeades, 1996; Bänzinger and Cooper, 2001; Bänzinger *et al.*, 2000). Physiology of maize shows that certain plant characteristics that are less relevant under non-stressed conditions become important for yield under drought and N stress. It is possible that other easy-to-use secondary traits could be identified and be employed to speed up breeding progress. However, close relationship between these traits and drought or low nitrogen will need to be established.

1.2 Objective of the study

The main objective of this study was to assess performance of a selected group of CIMMYT maize inbred lines to (selected from CML and CZL groups) achieve superior hybrids with tolerance to drought and low soil nitrogen stress environments.

1.3 Specific objectives:

- To determine the combining ability (GCA and SCA) of selected maize inbred lines for performance under drought stress and low nitrogen, including the interaction of GCA and SCA with environments,
- To determine the effects of low-N and drought stress on yield of single crosses (SCs) derived from crosses among selected maize inbred lines,
- To determine the correlation between grain yield and other traits, and
- To evaluate the patterns of G x E interactions of hybrids.

1.4. Hypotheses:

- There is no difference in performance among hybrids evaluated under drought and/ or low-N stress environments, and there is no interaction between combining ability and environments.
- The yields of single crosses (SCs) derived from the maize inbred lines under evaluation are not affected by low-N or drought stress.
- There is no relationship between grain yield and potential traits for indirect selection of grain yield under stress.
- The performance of SC hybrids under evaluation varies with environments in detectable patterns.

CHAPTER 2

LITTERATURE REVIEW

2.1 Effects of drought stress on maize

Drought can affect maize at different stages of development starting from crop establishment up to grain filling. The physiological responses of maize to drought are a complex phenomenon and often unpredictable (Frova *et al.*, 1999; Moreno *et al.*, 2005). Drought stress can affect the growth and yield of maize during the following three critical periods in the plant's growth; a) at crop establishment, b) at flowering, and c) during mid - to late grain filling stage. However, grain yield is most severely affected when drought stress occurs during the flowering and grain filling period (Bänzinger *et al.*, 2000; Grant *et al.*, 1989; Setter and Parra, 2010).

Under severe drought stress at flowering, the number of kernels and the anthesis silking interval (ASI) are affected (Bolaños and Edmeades 1996; Setter and Parra, 2010; Boyer and Westgate, 2004). Extreme sensitivity is seen during the period 2 to 22 days after silking, with a peak at 7 days. At the peak the number of kernels may be reduced to 45% of their potential and the anthesis-silking interval (ASI) is lengthened (Bolaños and Edmeades, 1993; Setter and Parra, 2010). When stressed in the period 12 to 16 days after silking, kernel weight may fall to 51% of potential weight. Drought stress in the interval from just before tassel emergence to the early grain filling stage may cause yield reductions up to 90%, and almost complete barrenness (NeSmith and Ritchie 1992; Grant *et al.* 1989; Setter and Parra, 2010).

Maize is thought to be more susceptible to drought stress at flowering than other rain-fed crops because of its botanical structure. Unlike other cereals, the male and female flowers in maize are also distantly separated (often around 1 m apart), and both the pollen and the fragile stigmatic tissue must be exposed to a desiccative environment for pollination to occur. The female florets in maize develop almost simultaneously and usually on a single ear on a single stem. Additionally, when drought reduces photosynthesis shortly before flowering, silk growth is delayed, leading to a very evident increase in the anthesis-silking interval (ASI) and a lack of pollination (Banziger *et al.*, 2000; Heisey and Edmeades, 1999)

Even though reasonable quantities of plant reserves are accumulated well before flowering and stored in the stem (during its first two weeks of ear development), the maize ear has very little capacity to access these reserves. That is why successful pollination in drought-stressed maize has often been followed by abortion of the kernels a few days later (Westgate and Boyer 1986; Setter and Parra, 2010). Drought diminishes the capacity of developing kernels to use available assimilates because the functioning of a key enzyme, acid invertase, is impaired (Westgate, 1997; Boyer and Westgate, 2004; Setter and Parra, 2010).

2.2. Effects of low soil nitrogen on maize yields

Maize has a strong positive response to nitrogen (N) supply, and inadequate N is second only to drought as a constraint to tropical maize production (Vance, 2001; Bänziger and Diallo, 2004). Nitrogen stress reduces photosynthesis by reducing leaf area development and accelerating leaf senescence.

The pattern of nitrogen stress is usually similar across locations. Nitrogen is particularly concentrated in leaves, especially in photosynthetic enzymes, where it may account for up to 4% of the dry weight. It is an essential component of chlorophyll, protein, and nucleic acids (Dackard *et al.*, 1989, Banziger *et al.*, 2000).

Most of the N present in soil (95 to 99%) is not readily available to plants, as it is bound up in the soil's organic matter (Banziger *et al.*, 2000). Nitrogen is available to maize and other non-leguminous plants as nitrate (NO_3^-) and ammonium (NH_4^+), known as mineral N. These are provided through gradual decomposition of organic matter in the soil, application of fertilizers, the release of ammonium ions from clay minerals, and slightly through rain. However, the predominant form available is nitrate, formed by the conversion of NH_4^+ to NO_3^- by nitrifying soil bacteria (Dackard *et al.*, 1989, Banziger *et al.*, 2000).

The morphological and physiological responses in maize due to a continuous N deficit include: diminished plant size, reduced efficiency in use of radiation, increased mobilization of vegetative N to the grain, and a lowered plant N concentration (Muchow and Davis, 1988; Vance, 2001). According to Presterl *et al.* (2002), Nitrogen-deficiency stress delays shoot elongation and leaf growth, but increases root growth. In addition, the

volume of roots is usually lower under N stress than under normal N fertilization. However, there is still not enough information on how low-N stress affects reproductive development, though initiation and development of the reproductive structures occur in distinct phases, each being affected by N stress. Severe N stress typically delays silking more than it does anthesis. This exaggerates the anthesis silking interval (ASI) under N stress at flowering. Like for drought, silking delay also results in poor pollination and, kernel and ear abortions under N stress. Nitrogen deficiency during grain filling accelerates leaf senescence and reduces the crop's rate of photosynthesis and its kernel weight. Pearson and Jacob (1987) and Bänziger, *et al.*, (2000) reported that yield in maize is affected by nitrogen supply more strongly before anthesis than after anthesis. Additionally, low-N stress induces premature leaf yellowing (first stage of senescence) but this process is reversible.

Nitrogen requirements for the maize crop can be met by addition of organic or inorganic fertilizer. However, the non-availability and high price of fertilizers contribute to constraints, limiting maize productivity in most developing countries as the majority smallholder farmers lack resources for purchasing yield-improving inputs. In spite of the maize yield potential of above 10 t/ha, fertilizer application on crops averages 25 kg/ha and seems to have decreased over the past 10 years as farmers have faced increasing input costs (FAOSTAT, 2003) and decreasing production prices (Bänziger *et al.*, 2004).

In a 2000-2002 survey of sub Saharan Africa, Larsson (2005) reported that 53% of smallholder farmers did not apply fertilizer at all during the 2002 season and most of those who did, used very small quantities averaging 14 kg/ha. These quantities are low

compared to what commercial farmers apply (300-400 kg/ha). Therefore, a breeding approach to develop stress tolerant varieties is highly recommended, to increase productivity under low input conditions

2.3 Breeding strategies for developing maize drought and low-N tolerant varieties

According to Banziger *et al.* (2000), breeding methodologies in the tropics were strongly influenced by maize breeding in temperate areas. In temperate environments maize is grown under relatively stress free conditions, and farm yields are comparable to yields obtained from experimental stations. However, in tropical environments maize is frequently stressed and on-farm yields fall far below those obtained on breeding stations. This means that selection under high yielding conditions may not be the best way to increase yields in farmers' fields.

2.3.1 Breeding approaches for drought tolerance

One approach in breeding maize for tolerance is to select cultivars that can escape this stress by maturing sufficiently early. Selection for earliness attempts to match the phenology of the maize variety to the pattern of water availability. However, earliness is often subject to a penalty in yield when the rainfall amount and duration is higher than average. Under those circumstances, the yield of an early maturing cultivar is limited by the amount of radiation the cultivar can capture - normally less than that of a later maturing cultivar (Banziger *et al.*, 2000).

The other approach is to breed for drought-tolerant maize genotypes. However, selection for both high yield and drought tolerance in maize is hampered by: (a) the lack of ability to control stress conditions, (b) yields that are too low or too high to be useful for selection when trials take place in uncontrolled, drought-prone environments, (c) high error variances that are typical when testing in drought-stressed environments, (d) the apparent negative correlation between drought resistance and yield potential of a hybrid when grown under favorable growth conditions, and (e) high G x E interactions resulting from different characteristics of genotypes, or from variations in rainfall and temperature from location to location and year to year. (Jensen and Cavalieri, 1983, Bänzinger and Cooper, 2001).

The International Maize and Wheat improvement Centre (CIMMYT) started to improve maize for drought tolerance in the 1970s (Edmeades *et al.*, 1989). Progenies of experimental maize were evaluated under three carefully managed water supply levels (1) flowering drought stress, (2) grain filling drought stress and (3) well watered conditions. Drought stress during flowering is the most severe to affect maize yield (Bänzinger *et al.* 2000; Bänzinger *et al.*, 2004). Selection was for an index that sought to maintain constant anthesis date and grain yield under well water conditions, increase grain yield stem and leaf extension under drought and decrease anthesis silking interval (ASI), leaf senescence and canopy temperature under drought (Bolanos and Edmeades, 1993a). Selection gains under drought were due to increased partitioning of dry matter to the growing ear, but biomass production and likely water uptake did not change (Edmeades *et al.*, 1993 and 1999; Monneveux *et al.*, 2006). Using a selection approach based on three types of

environments described as: recommended agronomic management/high rainfall condition, low-N stress and managed drought stress, Bänziger *et al.* (2004). Forty-one CIMMYT maize hybrids were compared in performance with forty-two released and pre-released hybrids from private seed companies in several environments across east and southern Africa. Hybrids from the CIMMYT stress breeding programme showed consistent advantage over commercial checks and hybrids from private companies at all yield levels. Those results suggested that simultaneous selection for tolerance and resistance to abiotic and biotic stress while monitoring performance under high potential conditions could result in significant progress for target environments where combination of stresses occur at lower yield level.

Physiology of maize shows that certain plant characteristics that are less relevant under non-stressed conditions become important for yield under drought and N stress. The most apparent example is the ability of a genotype to produce grain-bearing ears under drought stress at flowering. This characteristic can only be observed under stress conditions. This requires managing both drought and low-N tolerance stresses. In the case of drought, this is achieved by conducting experiments partly or entirely during dry seasons and managing the stress through irrigation. In the case of low-N, this is achieved by carrying out experiments in fields that are depleted of nitrogen. The objective of such experiments is to measure the genotypic drought tolerance and/ or the genotypic low-N tolerance (Bänziger *et al.*, 2000; Musila *et al.*, 2010).

Most assessments on progress of CIMMYT's drought maize populations were conducted in environments where the populations were selected and it was hypothesized that selection gains may be limited to particular drought conditions in the selection environment (Bänziger *et al.*, 2004). Greater yield stability of one drought tolerant selected population compared to its conventionally selected counterpart has been achieved across international testing locations. Improvements under drought were associated with selection gains across a wide range of nitrogen supply levels (Bänziger *et al.*, 2000) indicating that the screening approach using managed drought environments may have wide merits (Bänziger *et al.*, 2004).

2.3.2. Breeding approaches for low-N tolerance

A significant proportion of all maize in the tropics is produced under low-N condition. Many small holder farmers either do not gain the return from N use necessary to justify its cost, or they cannot afford or even access it. In addition, production of organic manure is not efficient for these farmers. Breeding for the plant's efficient use of N is possibly an effective strategy to reduce the farmer's cost of fertilizer, developing maize genotypes with both highly efficient use of nitrogen and high yield potential (Joseph *et al.*, 2001, Meseke *et al.*, 2006). Results of research indicated that maize varieties vary in performance according to soil fertility levels and the plant's effectiveness in using N. (Lafitte and Edmeades, 1994; Scharf *et al.*, 2002). The high-yielding commercial maize varieties generally released in the tropics are selected under optimum N fertility

conditions and may not serve the interests of resource-poor farmers who cannot afford inorganic fertilizer. Hence, the need to develop maize varieties tolerant to low-N levels.

The main challenge in breeding for low-N tolerance is to guarantee successful selection. A breeder not only needs the right source germplasm but also be able to evaluate N tolerance precisely under conditions that are relevant to the target environment and selection intensity. This requires a good understanding of the crop's behavior under a low-N environment, ability to impose appropriate levels of stress, a suite of secondary traits related to yield under stress, adequate statistical designs to use during selection, and an appropriate choice of both germplasm and breeding schemes (Bänziger *et al.*, 2000).

Breeding for tolerance to low-N stress is a bit simpler than for tolerance to drought, because N deficiency usually affects plant growth more evenly over time than do random drought spell (Bänziger *et al.* 2000). Consequently, testing lines under one level of relatively severe stress for low-N should be adequate to access low-N stress tolerance for various levels of N deficiency. Various studies have revealed that genotypes selected for tolerance to drought can also perform well under low-N conditions. Lafitte and Edmeades (1995), Lafitte and Bänziger (1997), Logrono and Lothrop (1997), and Bänziger *et al.* (1999) have reported good performance under low-N conditions of tropical maize genotypes that were selected for drought tolerance. Others have observed that recurrent selection under drought has a spillover effect on performance under low-N conditions (Bänziger *et al.* 1999). Additionally, Lafitte and Bänziger (1997) found four maize populations (Tuxpino Sequia C8, Laposta Sequia C3, Pool 26 Sequia and Pool 18 C3)

that combined tolerance to both drought and low-N. Zambezi and Mwambula (1997) reported that improvement of drought tolerance in a maize population, Tuxpino Sequia, resulted in improved performance under low-N conditions.

One approach to reducing the impact of N deficiency on maize production is to select cultivars that use available N more effectively, due either to their enhanced capacity for its uptake or their more efficient use of absorbed N in producing the grain (Lafitte and Edmeades, 1994a). It has been suggested that selecting for yield in the target environment is more effective than selecting for yield potential alone (Bänziger *et al.*, 2000). However, maize breeders do not like these compromised environments due to their increased variability as soil fertility declines, and thus a decline in heritability for yield (Lafitte and Edmeades, 1994a). However, they suggest that a reasonable strategy for breeding productive maize under N-limited conditions would be to develop early- and late-maturing source populations that are able to take up large quantities of N, partition a large proportion of plant N to the reproductive structures (high N harvest index), and maintain a high N concentration in the grain under a limited N supply.

The benefit of secondary traits in selecting for tolerance to low-N has been demonstrated (Bänziger *et al.*, 2000). This study suggested the use of a selection index in which grain yield is weighted at 50%, ears per plant and leaf senescence at 20% each, and ASI at 10%. In comparison, for drought tolerance these authors recommended weighing grain yield at 33%, ears per plant at 20%, leaf senescence, tassel size, and ASI at 13% each, and leaf rolling at 7%.

2.4 Combining ability

In a hybrid development program, the main objective is to identify a new line when combined with other lines to produce high performing hybrids. If resources were not limited, the best way is to test immediately each new inbred line in combination with every other inbred with which it could be a parent in a hybrid cultivar. However, due to a large number of single-cross combinations that would have to be tested, this is not feasible. Therefore, the breeder must identify a limited number on inbred lines having sufficient genetic potential prior their evaluation in specific hybrids combinations (Walter, 1987).

The concept of combining ability was introduced by Sprague and Tatum (1942) who defined it as the potential of parents to produce superior progenies following hybridization. Shattuck *et al.*, (1993) also defined it as the magnitude of additive and non-additive gene action. The concept of combining ability has become increasingly used in plant and animal breeding and especially it is useful in connection with testing procedures, where it helps to study and compare the performance of a given parent in hybrid combination (Griffing, 1956). Combining ability comprises, a general combining ability (GCA) that is used to designate the average performance of a line in hybrid combination, and specific combining ability (SCA) used to designate situations where certain crosses excel relatively better or do worse than expected based on the average performance of the lines involved. A parent with a GCA estimate of 0 has an average combining ability and depending on the index used, parents with positive or negative GCA values perform above or below average. SCA expresses the performance of the

progeny from a cross between two parents based on the average performance of the parents involved. SCA estimates are either positive or negative. GCA is attributed to additive gene action and SCA to non-additive effects (Shattuck *et al.*, 1993).

Diallel analysis is known to be used in estimating GCA and SCA effects and their implications in breeding (Griffing, 1956; Baker, 1978). In diallel analysis parents are crossed in pairs where each parent is crossed with every other parent. Griffing (1956) proposed four diallel mating designs for generating and analyzing diallel crosses. He also demonstrated a statistical analysis of data generated from all the four diallel mating designs for estimating GCA and SCA variances and effects (Griffing, 1956). Design I consists of a full diallel comprising all parents (selfing), F1 crosses, and reciprocals crosses; design II is a full diallel comprising only parents and crosses without reciprocals. The third design comprises only both forward and backward crosses without parent and the last one comprises only the forward crosses.

Two important assumptions (fixed and random models) regarding parents involved in the mating are considered prior producing crosses and their evaluation Griffing (1956). If the parents are deliberately selected, then Model One (fixed) is appropriate and inferences should be restricted to the set of parents evaluated. The model II (random) is considered when the evaluated parents are used as a sample to obtain information on the population from which they were selected.

The method and model selected can affect data interpretations. Therefore, the accuracy of the analysis is improved by using the appropriate method and model. However, the

program objectives and parental sampling procedures will determine the most appropriate model to use (Shattuck *et al.*, 1993).

General combining ability (GCA) and SCA can interact with the environment and cause changes in expected parental combining abilities over the environments (Singh *et al.*, 1992). Therefore, to obtain precise combining ability estimates, it may be necessary to evaluate parents in more than one environment. When the diallel is conducted in only one environment, attempt should be made to match the diallel with the environment of interest (Shattuck *et al.*, 1993). Betrán *et al.*, (2003b) evaluated seventeen maize inbred lines crossed in a diallel design under stress and non-stress environments and reported significant GCA and GCA x environment interaction effects for grain yield

2.5 Relationships between grain yield and key secondary traits under stress

Most maize in the tropics and especially in sub-Saharan Africa is grown under stress environments (Bänziger *et al.*, 1997; Diallo *et al.*, 2001). This raises the need of efficient breeding strategies for such conditions. Breeders are primarily interested in grain yield; however, heritability that determine genetic advance under selection can change with stress. Additionally, Bolaños and Edmeades (1996) reported that selection for improved performance under stress based only on grain yield has often been considered inefficient, but the use of secondary traits of adaptive value whose genetic variability increases under stress can increase selection efficiency.

Secondary traits can be useful in that they can improve the precision with which drought or low-N tolerant genotypes are identified, compared to measuring grain yield under alone drought or low-N stress. This is because under stress the heritability of grain yield usually decreases, whereas the heritability of some secondary traits remains high, while at the same time the correlation between grain yield and those traits increases sharply (Bänzinger and Lafitte 1997a; Bolaños and Edmeades 1996; Bänzinger *et al.*, 2000).

A small number of secondary traits have been used in breeding programs and even fewer have proven to contribute to the improvement of drought or low-N tolerance in maize (Bänzinger *et al.*, 2000). These authors pointed out major secondary traits that are useful for selection apart from grain yield alone under stress; ears per plant, anthesis-silking interval, leaf senescence. Similarly, Bolaños and Edmeades (1996), found correlations with different various signs between drought tolerance with ears per plant, days to silking, ASI, leaf rolling, tassel size and plant height.

2.6 Genotype x environment interaction

Phenotype is a joint contribution of both genes and environment (Chahal and Gosal, 2002). Additionally, the effect of a given environment is not exactly the same on the performance of different genotypes. A genotype may be superior to another in one environment, equal in second environment but inferior to it in another environment. This kind of variation resulting from differential performance of genotypes in different environments leading to change in rank or even relative performance is known as the genotype environment (GE) interaction (Chahal and Gosal, 2002). This difference in

expression of genotypes across environments decreases association between genotypic and phenotypic value hence the difference in performance between a set of genotypes when evaluated across environments. Given the performance of any genotype is conditioned by environment it makes difficult to predict the performance of a genotype in any environment, the well performing candidates in one environment may perform very poorly in another (Chahal and Gosal, 2002; Ntawuruhunga *et al.*, 2001).

Because of the effect of G x E, maize genotypes need to be screened under many environments in order to determine their real genetic potential. The screening can be done under an artificial environment, for example, different levels or combinations of fertilizer, or under a natural environment (different locations or seasons) or both combined. (Ntawuruhunga *et al.*, 2001; Chahal and Gosal, 2002). The G x E interactions also determines the types of variety that can be developed and the range of environments under which selection should be carried out.

Thus, G x E interactions influence not only the breeding objectives but also the breeding strategies which may be aimed either at exploiting or avoidance of these types of interactions. For example, varieties that exhibit good performance across several environments can be deployed for use across a wide range of agro-ecologies while others have limited high performance environments and therefore used only for a particular agro-ecology. Decisions influenced by existence of G x E interactions include the type of adaptation to bred for, choice of location for early generation selection, type of environments (stress or non-stress) and the range of environments for final evaluation

before the release of varieties (Chahal and Gosal, 2002). G x E interactions therefore is not only constraining farmers but also researchers as it slows down breeding progress. The problem could, however, be eased through developing genotypes with a wide adaptation to target environments.

Drought and low-N are the most important environmental constraints to yield stability. Extensive testing of maize hybrids improved for drought conditions under low-N and drought stress as well as in optimal growing environments would be useful for identifying hybrids that combine high yield and least G x E interactions (Yan and Kang, 2003; Meseke *et al.*, 2008). Eberhart and Russell (1969) demonstrated that heterogeneous maize populations have better yield stability than homozygous populations, and further observed that two single cross hybrids were not only as stable as any of double crosses but also higher yielding.

The detection and quantification of G x E interactions and stability has been attempted through several statistical models (Chahal and Gosal, 2002; Lin *et al.*, 1986). However, regression analysis has been commonly used for estimating yield stability in plant breeding programs (Eberhart and Russell, 1966). It partitions the G x E interactions (GEIs) into GE and residuals across varying environments in order to provide a meaningful biological explanation of GEIs (Eberhart and Russell, 1966; Avis *et al.*, 1980). Regression approaches have been used widely for partitions the G x E interactions. However, for many yield trials, regression models usually fail to capture much of the interaction sums of squares (Gauch, 1992). Additive Main Effects and

Multiplicative Interaction (AMMI) has now become a method of choice for GEI analysis and interpretation (Ntawuruhunga *et al.*, 2001; Gauch and Zobel, 1988). It is a combination of analysis of variance and the Principal Component Analysis (PCA). The distinctive aspect of AMMI model is the use of biplot which helps to visualize relationship between eigenvalues for PCA and genotypic and environment means (Gauch, 2006).

The major advantages of AMMI model over regression linear models is that the interaction is allocated into many multiplicative parameters which are independent of each other. It precisely rank yield across environments and then partition the data into pattern and noise and its graphical representation of the data with the biplot reveals more pattern of GEI (Ntawuruhunga *et al.*, 2001; Gauch, 1992).

Similarly, among new statistical tools currently used to detect and quantify G x E interactions, the use of genotype main effect (G) and genotype-by-environment (GE) interaction (GGE) biplot analysis is recommended (Yan *et al.*, 2007). GGE biplot analysis can provide comprehensive visual information and is better, faster and easier to interpret than the results obtained from regression analysis (Yan and Kang, 2003). The GGE biplot analysis removes the large environmental effect (E), which is irrelevant to genotype evaluation, and keeps only G and G x E that are relevant for making meaningful genotype evaluation and selection decisions (Yan *et al.*, 2001; Yan and Kang, 2003).

Recently, however, GGE legitimacy was questioned by a proponent of AMMI analysis but Yan *et al.*, (2007) concluded that both GGE biplot analysis and AMMI analysis combine rather than separate G and G x E in mega-environment analysis and genotype evaluation. Secondly, the GGE biplot is superior to the AMMI graph in mega-environment analysis and genotype evaluation because it explains more G+GE and has the inner-product property of the biplot. Third, the discriminating power vs. representativeness view of the GGE biplot is effective in evaluating test environments, which is not possible in AMMI analysis. The combination of GGE-biplot and AMMI is used in this study.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Germplasm source

Twelve maize inbred lines of tropical origin that differed in their response to drought stress were used in this study. The elite inbred lines (CZLs) were developed by Breeding Programs at CIMMYT-Zimbabwe but not officially released as CMLs. Detailed description of these inbred lines is provided in Table 1. Five of these inbred lines were known drought tolerant sources (T), while the other seven were elite inbred lines (E) were unknown tolerance to drought and low N (Table 1). Some of these inbreds (CML group) have been used as testers (CML 78, CML 341, CML 379, and CML 499) by various research programs to assess new experimental lines and classify them into heterotic groups. Checks were chosen based on response to managed stressed environment, maturity and their yield.

3.2 Experimental design

Half-diallel crosses were conducted between the twelve parents in 2008 and 2009 at National Crops Resources Research Institute (NaCRRI), in Uganda. All experiments were planted in an alpha-lattice design (Patterson and Williams, 1976) in one row plots and replicated twice. The inbred lines were crossed by hand pollination in all possible forward combinations without reciprocals. Pollen control was achieved through bagging

the maize ears appropriately. Plants within a family line that appeared to be off types (i.e. with severe disease symptoms or malformations), were not used in the crossing.

Of the 66 crosses expected from each of the three seasons, 59, 53 and 57 single-cross (SC) hybrids were generated, respectively, in seasons 2008A, 2008B and 2009A (Table 2). These hybrids were subjected to evaluation in the season that followed, i.e. 2008B, 2009A and 2009B, respectively, with appropriate checks included in each trial.

Table 1: Description and pedigree of maize inbred lines used in diallel study to evaluate performance under drought and low- nitrogen stress conditions at NaCRRI-Uganda and KARI (Kiboko)-Kenya

Genotype	Category†	Pedigree	Classification
<u>Inbred Line</u>			
CZL 00003	E	-	Mid-altitude
CZL 00034	E	-	Mid-altitude
CZL 02004	E	-	Mid-altitude
CZL 02008	E	-	Mid-altitude
CZL 02009	E	-	Mid-altitude
CZL 01001	E	-	Mid-altitude
CZL 03002	E	-	Mid-altitude
	T	(CL-04345*CML274)B-15-1-2-	Lowland
CML 499		B*4	
	T	Pob21C5HC218-2-3-B-###-B-1-	Lowland
CML 258		BBB-f	
CML 341	T	LPSEQC3-H1-2-2-2-1-1-##-B-B	Lowland
CML 78	T	P.32 C19MH32-1-#2-B-###-3-B	Sub-tropical
CML 379	T	P501c1#-303-1-1-1-2-1-3-B	Sub-tropical
<u>Checks</u>			
CML 202/CML 395	-	Commercial hybrid	Mid-altitude
H 513	-	Commercial hybrid	Mid-altitude
Longe 2H	-	Commercial hybrid	Mid-altitude
Longe 6H	-	Commercial hybrid	Mid-altitude
Longe 10H	-	Commercial hybrid	Mid-altitude
DK 8031	-	Commercial hybrid	Mid-altitude

† T, Tolerant lines; E, Elite lines.

CZL, CIMMYT Zimbabwe Line; CML, CIMMYT, Mexico, line.

NaCRRI, National Crops Resources Research Institute

Table 2: Single-cross hybrids generated from selected parents

Parents	08A	08B	09A	Combined
CZL 00003	11	9	11	9
CZL 00034	11	9	7	5
CZL 02004	10	8	11	8
CZL 02008	10	8	9	7
CZL 02009	8	8	9	6
CZL 01001	11	11	10	10
CZL 03002	9	10	11	8
CML 499	8	7	10	4
CML 258	9	7	6	4
CML 341	9	10	9	6
CML 78	11	10	11	10
CML 379	11	9	10	9
Total	59	53	57	43

3.3 Environments and sites management

In all, 53-59 single-crosses were evaluated within different managed environment and 43 across managed environments at two sites, namely, NaCRRI-Namulonge and KARI-Kiboko Station. The following growing conditions were used in these sites

- (i) Managed drought stress conditions (Kiboko, KBMD)
- (ii) Well-watered conditions ((Kiboko, (KBWW) and NaCRRI, (NMWW))
- (iii) Low nitrogen stress conditions ((NaCRRI, (NMLN))
- (iv) Optimal nitrogen conditions (NaCRRI, (NMON)).

Details of the trial environment are summarized in Table 3

Table 3: Locations and environments used for evaluation

Location	Stress management and season
NaCRRI (Namulonge, Uganda, 00 32" N, 32° 37" E, 1150 masl)	
	Low-N- stress 09A, NMLN 09A
	Low-N- stress 09B, NMLN 09B
	Optimum N 09A , NMON 09A
	Optimum N 09A , NMON 09B
	Well-watered 08B, NMWW 08B
KARI (Kiboko, Kenya, 2°25''S, 37°75''E, 975masl)	
	Managed drought stress 08B, KBMD 08B
	Managed drought stress 09A, KBMD 09A
	Well-watered 09B, KBWW 09B

KARI = Kenya Agriculture Research Institute; NaCRRI = National Crops Resources and Research Institute; masl = meters above sea level.

Water stress was achieved at KARI, Kiboko by withholding water from 2 weeks before anthesis, while well watered conditions were achieved by irrigating every 3 days to field capacity until the completion of the crop's lifecycle (Bänziger *et al.*, 2000).

Low-N conditions have been established at NaCRRI over several years by depleting the soil of N in one field until it reached at least yield levels that are about 25 to 35% of those obtained under well-fertilized conditions as recommended by Bänziger *et al.*, (2000). To accomplish this, maize or sorghum have been cropped continuously for twenty years

without N fertilizer application, and all stover biomass has been removed after harvest. However, phosphorus (P, 60 kg/ha) and potassium (K, 30kg/ha) were applied, according to recommendations for the site.

Optimal N conditions were maintained by application of N, P, and K fertilizers at rates of 80 kg/ha, 60 kg/ha and 30 kg/ha, respectively. Prior to planting or the application of N, a soil analysis was performed to determine the initial level of N at the trial sites (Table 4). In both sites the soil texture was clay, and all the nutrients analyzed were at least at the critical level except for soil pH (4.6) in the site for optimum nitrogen conditions and P (3.6 ppm) in the low-N site.

Table 4: Soil characteristics at nitrogen trial sites at the National Agricultural Crop Resources Research Institute (NaCRRI, Namulonge, Uganda)

Site	pH	Organic matter	N	P	Ca	Mg	K	Texture
		-----%-----		-----ppm-----				
Optimum N	4.6	3.8	0.21	5.4	1221	302	353	Clay
Low-N	5.3	3.7	0.20	3.6	2175	389	760	Clay
Critical values	5.2	3.0	0.20	5.0	350	100	150	
Sufficient levels	7.0	6.0	0.3	20.0	2000		500	

3.4 Field lay-out and trial management

The field trials were laid out in an alpha-lattice design with two replications. Each replication consisted of one-row plots, with the assumption that inter-genotypic competition from single-row plots is unlikely to substantially affect the results (Bänziger

et al., 1995). The plots were 4.0m in length at Kiboko, and 5.0m at Namulonge with 0.75m between rows in all experiments, while the inter-hill spacing was 0.20m and 0.30m, respectively at Kiboko and Namulonge

At each location, maize genotypes of similar vigor were used as a border, and appropriate checks were included for comparisons (Table 1). Two seeds per hill were planted along each row, and thinned to one plant per hill about two to three weeks after emergence. Whenever necessary, supplemental irrigation was provided at NaCRRI (NMLN & NMON). Termiticide sprays (Chlorpyrifos, 480g/l) were applied when necessary to control termites that were problematic at these sites. Other standard cultural and agronomic practices were followed in managing the trials. Greater alley effect under stressed environments was controlled; at harvest one plant at each end of each plot was excluded from trials, and yield /ha was adjusted to harvested area.

Under managed drought conditions, water was supplied with overhead sprinklers every 5 days to field capacity during vegetative growth. Drought stress around flowering time to target grain filling stages was achieved by withholding water from 2 weeks before anthesis. However, in order to fill kernels that were already developing under stress, a rescue irrigation was applied one month after flowering (Bänziger *et al.*, 2000). The crop completed its remaining cycle without further irrigation or rain. To achieve well-watered conditions at Kibobo, where rainfall was rare, irrigation with overhead sprinklers was applied every 3 days to field capacity until completion of the crop's lifecycle.

The NaCRRRI well-watered site (NMWW) differed from NMON only in that it was one season earlier and did not have a corresponding experiment grown under low-N conditions (NMLN).

3.5 Field measurements

Data was recorded on plot basis on the following agronomic traits: days to anthesis (AD) (number of days from planting to 50% of plants having begun pollen shed), days to silking (SD) (number of days from planting to 50% of plants showing silk emergence). The anthesis-silking interval (ASI) was calculated as the difference between silking and anthesis dates ($ASI = SD - AD$). Leaf senescence (Lsene), was scored following methods described by Bänziger *et al.*, (2000). Leaf senescence was scored during grain filling on a scale of 0-10 by dividing by 10 the percentage of estimated total leaf area that had turned brown (dead leaf). Thus, a score of 1 = 10%, 2 = 20%, 3 = 30%, and so on. Leaf yellowing (Lyelw) was measured on a scale of 1-5, with 1 being leaves that remain green and 5 all yellowish leaves (Personal communication, Asea, G., 2009).

Ears per plant (EPP) were determined as the number of ears with at least one fully developed grain, expressed as a fraction of the number of plants at harvest. Under the stressed environments, the shelled grain weight (GW) was used to calculate grain yield ($t\ ha^{-1}$) adjusted to 12.5% grain moisture (Bänziger *et al.*, 2000). In all experiments under optimum conditions, field weight (FW, weight of the harvested ears) was multiplied by 0.80 shelling percentage to obtain grain yield ($t\ ha^{-1}$), adjusted to 12.5% grain moisture.

Grain moisture (g kg^{-1} moisture) at harvest was measured, using either a separate moisture meter or computerized equipment mounted on the combine manufactured by Almaco company.

3.6 Data analysis

Analysis of variance for individual environments was conducted based on the lattice design using GENSTAT V12 (Genstat, 1010). Genotypes and imposed management environments (stressed or optimal conditions) were considered to be fixed effects and replications and blocks within replications were considered random effects. Combined analyses were done based on common hybrids in all environments and analyses were computed across related environments, and across all environments, using the general analysis of variance procedure in GENSTAT. Genotype means within and across environments were compared using Fisher's protected LSD.

3.6.1 Diallel analysis of single crosses

Lattice-adjusted means were used to estimate the general combining ability (GCA) effects of the parents and the specific combining ability (SCA) effects of the crosses, based on Griffing's Diallel Method 4 (crosses only, without reciprocals or parents) and Model I (fixed effects) (Griffing, 1956). The analysis was computed by the GENSTAT Generalized Linear Model (GLM) regression procedure. To obtain the regression, a variable was created for each parent, with a value of 1 if that parent was included in the cross being considered, and a value of 0 otherwise. GCA estimates were calculated from

parameter estimates supplied by the GLM procedure, taking into account the number of crosses that involved each parent. Standard errors of GCA estimates were calculated according to the usual formulas (Griffing, 1956), then adjusted upward to reflect the number of crosses missing for that parent, using a formula supplied by Prof Paul Gibson (personal communication, June, 2010). GCA variance components and their interactions with environment were also adjusted according to the number of crosses (Gibson, P., personal communication, June, 2010). SCA estimates were obtained as the difference between the observed mean for a cross and the predicted mean obtained by GLM from the GCA estimates. Standard errors of SCA estimates were calculated according to the usual formulas (Griffing, 1956).

The significance of SCA and GCA sources of variation was computed using the lattice effective error variance in single environments, and the corresponding interaction of GCA or SCA with environments in multi-environment analyses. The significance of SCA x environment and GCA x environment interactions was determined using the pooled lattice effective error. SCA and GCA variance components were determined assuming a fixed model for the diallel. The relative importance of additive and non-additive effects (GCA and SCA, respectively) was estimated according to Baker (1978) as the ratio $2 \sigma^2_{GCA} / (2\sigma^2_{GCA} + \sigma^2_{SCA})$, where σ^2_{GCA} and σ^2_{SCA} are the estimated GCA and SCA variance components, respectively. The relative magnitude of heritable variation compared to total variation in a set of fixed parents was computed as the broad sense (BS) and narrow sense (NS) coefficients of genetic determination (CGD). For individual environments, NS CGD = $2 \sigma^2_{GCA} / (2\sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_e)$, while BS CGD = $(2 \sigma^2_{GCA} + \sigma^2_{SCA}) / (2\sigma^2_{GCA} + \sigma^2_{SCA} +$

σ^2_e). For combined environments, the estimates are similar, but have G x E components in the denominator. Means and magnitudes of genetic variances estimated in stressed environments were compared with those from non-stressed environments. Individual GCA and SCA effects were tested by a two-sided t-test to determine if they differed significantly from 0, based on the standard error associated with that effect. Skeleton ANOVA's for anticipated results and the formulas for calculating variance components and their contribution to phenotypic variance are summarized in Tables 5 to 9, below.

Table 5: Skeleton ANOVA for within environments analysis

Source	Type of Effect	df	†Expected mean squares	F-test denominator
Rep	Random	1		Rep.Block
Rep.Block	Random	5-11		Error
Crosses	Fixed	52-58	$\sigma^2_e + 2 \times \sigma^2_{\text{Cross}}$	Error
‡GCA	Fixed	11	$\sigma^2_e + 10 \times (c/66) \times \sigma^2_{\text{gca}}$	Error
SCA	Fixed	41-47	$\sigma^2_{e+} \sigma^2_{\text{sca}}$	Error
Lattice effective Error		30-44	σ^2_e	

† Expected mean squares are on an entry-mean within-environment basis, thus matching the tables presented in chapter four. The error term in ANOVA results was divided by the number of reps (=2) in order for it to correspond with the other mean squares.

σ^2_e = error variance component; σ^2_{sca} , σ^2_{gca} = general and specific combining ability variance component; σ^2_{Cross} = Cross variance component; σ^2_{Block} = .variance component.

‡ “c” represents the number of crosses out of the complete number (66), and varied from 53-59, depending on the trial.

Table 6: Skeleton ANOVA for across environments analysis

Source	Type of Effect	df	Expected mean squares	F-test denominator
E	Random	1		Rep/E
Rep/E	Random	2		PE
Crosse(C)	Fixed	42	$\sigma^2_e + 2 \times \sigma^2_{c \times e}$	E x C
GCA	Fixed	11	$\sigma^2_e + 10 \times (43/66) \times \sigma^2_{gca \times e} + 2 \times 10 \times (43/66) \times \sigma^2_{gca}$	GCA x E
SCA	Fixed	31	$\sigma^2_e + \sigma^2_{sca \times e} + 2 \times \sigma^2_{sca}$	SCA x E
E x C	Fixed	42	$\sigma^2_e + \sigma^2_{c \times e}$	PE
GCA x E	Fixed	11	$\sigma^2_e + 10 \times (43/66) \times \sigma^2_{gca \times e}$	PE
SCA x E	Fixed	31	$\sigma^2_e + \sigma^2_{sca \times e}$	PE
P E		62	σ^2_e	

E (e) = Environment; S (s) = Season; C (c) = cross; PE = Pooled Error.

σ^2_e = error variance component; $\sigma^2_{sca \times e}$ and $\sigma^2_{gca \times e}$ = general and specific combining ability by environment variance component; $\sigma^2_{c \times e}$ = Cross by environment variance component.

Table 7: Skeleton ANOVA for across seasons analysis

Source	Type of Effect	df	Expected mean squares	F-test denominator
S	Random	1		Rep/S
Rep/S	Random	2		PE
C	Fixed	42	$\sigma^2_e + \sigma^2_{c \times s} + 2 \times \sigma^2_c$	S x C
GCA	Fixed	11	$\sigma^2_e + 10 \times (43/66) \times \sigma^2_{gca \times s} + 2 \times 10 \times (43/66) \times \sigma^2_{gca}$	GCA x S
SCA	Fixed	31	$\sigma^2_e + \sigma^2_{sca \times s} + 2 \times \sigma^2_{sca}$	SCA x S
S x C	Random	42	$\sigma^2_e + \sigma^2_{c \times s}$	PE
GCA x S	Random	11	$\sigma^2_e + 10 \times (43/66) \times \sigma^2_{gca \times s}$	PE
SCA x S	Random	31	$\sigma^2_e + \sigma^2_{sca \times s}$	PE
PE		79	σ^2_e	

E (e) = Environment; S (s) = Season; C (c) = cross; PE = Pooled Error; σ^2 = variance component

σ^2_e = error variance component; $\sigma^2_{sca \times s}$ and $\sigma^2_{gca \times s}$ = general and specific combining ability by season variance component; $\sigma^2_{c \times s}$ = Cross by season variance component.

Table 8: Skeleton ANOVA for across all environments analysis

Source	Type of Effect	Df	Expected mean squares	F-test denominator
E	Fixed	4		PE
E x S	Random	3		Rep/E
Rep/E	Random	8		PE
C	Fixed	42	$\sigma^2e + \sigma^2c \times s/e + 8 \times \sigma^2c$	GCA x E x S
GCA	Fixed	11	$\sigma^2e + 10 \times c/66 \times \sigma^2gca \times s/e + 8 \times 10 \times c/66 \times \sigma^2gca$	GCA x E x S
SCA	Fixed	31	$\sigma^2e + \sigma^2scaxs/e + 8 \times \sigma^2sca$	S
C x E	Fixed	168	$\sigma^2e + \sigma^2c \times s/e + 2 \times \sigma^2c$	SCA x E x S
GCA x E	Fixed	44	$\sigma^2e + 10 \times c/66 \times \sigma^2gca \times s/e + 2 \times 10 \times c/66 \times \sigma^2gca$	S
SCA x E	Fixed	124	$\sigma^2e + \sigma^2sca \times s/e + 2 \times \sigma^2sca$	SCA x E x S
C x E x S	Random	126	$\sigma^2e + \sigma^2c \times s/e$	S
GCA x E x S	Random	33	$\sigma^2e + 10 \times c/66 \times \sigma^2gca \times s/e$	PE
SCA x E x S	Random	93	$\sigma^2e + \sigma^2sca \times s/e$	PE
PE		297	σ^2e	

E (e) = Environment; S (s) = Season; C (c) = cross; PE = Pooled Error; σ^2 = variance component

Table 9: Formula of variance components contributing to phenotypic variance for grain yield

Component	Across WW	Component	Across MD (or LN or ON)
Hybrids (H)	$(MS H - MS H \times E)/2$	Hybrids (H)	$(MS H - MS H \times S)/2$
H x E	$(MS H \times E - PE)/2$	H x S	$(MS H \times S - PE)/2$
Component	Across Stress	Component	Across Non Stress
Hybrids (H)	$(MS H - MS H \times SM \times S)/4$	Hybrids (H)	$(MS H - MS H \times OM \times S)/4$
H x SM	$(MS H \times SM - MS H \times SM \times S)/2$	H x OM	$(MS H \times OM - MS H \times OM \times S)/2$
H x SM x S	$(MS H \times SM \times S - PE)/1$	H x OM x S	$(MS H \times OM \times S - PE)/1$
Component	Across W level	Component	Across N level
Hybrids (H)	$(MS H - MS H \times WM \times S)/4$	Hybrids (H)	$(MS H - MS H \times S)/4$
H x WM	$(MS H \times WM - MS H \times WM \times S)/2$	H x S	$(MS H \times S - PE)/2$
H* S/WM [‡]	$(MS H \times WM \times S - PE)/1$	H x E	$(MS H \times E - MS H \times S \times E)/2$
		H x S x E	$(MS H \times S \times E - PE)/1$
Component	All		
Hybrids (H)	$(MS H - MS H \times E \times S)/4$		
H x E	$(MS H \times E - MS H \times S \times E)/2$		
H x S x E	$(MS H \times S \times E - PE)/1$		

H = Hybrid; E= Environment; S = Season; MS = Mean Square; PE = Pooled Error; WW = Well-Watered; MD = Managed Drought; LN = Low-N; ON = Optimum N; SM = Stress management; WM = Watering Management; OM = Optimum Management.

[‡]The WW environment was planted at two locations, in different seasons, so in all analyses involving the WW environment, seasons were analyzed as a nested effect within environment.

3.6.2 Correlations between traits

Genotype means were used to compute Pearson correlation coefficients between traits within individual experiments, across seasons in the same environment, across related environments combined and all environments combined.

3.6.3 G x E analysis

To determine the implication of G x E interaction on yield, the ratio of various components contributing to the total phenotypic variance was determined. Thereafter, the Additive Main effects and Multiplicative Interaction (AMMI) (Gabriel, 1971) analysis was carried out to assess the relationship among genotypes and environments and to point out relatively stable genotypes. This was complemented by the use of genotype main effect (G) and genotype-by-environment (G x E) interaction (GGE) biplot analysis (Yan *et al.*, 2007). Genotype means were averaged across the two seasons per environment except where an environment was represented by only one season (KBWW and NMWW).

CHAPTER FOUR

RESULTS

4.1 Diallel analysis of single-cross performance in stress and non-stress environments

Grain yield varied significantly among the hybrids in all environments ($P < 0.001-0.1$) except Namulonge low nitrogen stress during the first season of 2009 (09A season) (Table 10). Low grain yield (1.1 to 3.5 t/ha) was observed under stress environments and the lowest means occurred under drought stress (1.6 and 1.1 t/ha during 08B and 09A seasons, respectively). High grain yields were observed in koboko well-watered (mean=7.7, max=12.8 t/ha) and high means were generally realized under optimum environments. The mean yield under drought conditions was 1.4 t/ha which is 23 % of grain yield under well watered conditions. Similarly, the mean yield under low-N stress was 2.7 t/ha which is the 50 % of grain yield under optimal nitrogen conditions. The highest yielding hybrid (CZL 00003/CML 341) under well watered conditions yielded 7.9 t/ha and the highest (CZL 00003/CML 499) under optimum nitrogen conditions yielded 6.5 t/ha compared to the best check (CML 202/CML 395) that yielded 1.1 t/ha under drought conditions.

Table 10: Analysis of variance for grain yield (t/ha) in individual testing environments

Sources of variation	df	NMLN	NMLN	KBMD	KBMD	NMON	NMON	KBWW	NMWW
		<u>09A</u> MS [†]	<u>09B</u> MS	<u>08B</u> MS	<u>09A</u> MS	<u>09A</u> MS	<u>09B</u> MS	<u>09B</u> MS	<u>08B</u> MS
Rep	1	8.03*	90.14***	0.34	0.34	14.65 ⁺	33.13***	2.19	8.19***
Rep.Block	5-11	0.81*	1.59**	0.76*	3.16***	3.34*	0.71	2.16	0.55
Hybrids(H)	52-58	0.25	0.83**	0.51**	0.50***	0.62 ⁺	0.59*	3.97***	0.58**
GCA	11	0.41	0.81*	1.85***	0.91***	0.76*	0.96**	5.52***	1.42***
SCA	41-47	0.20	0.84**	0.19	0.39*	0.58*	0.50	3.56***	0.38 ⁺
Error ‡	30-44	0.24	0.34	0.21	0.20	0.36	0.32	0.85	0.23
Cv		0.36	0.23	0.40	0.58	0.16	0.14	0.17	0.15
Mean		1.9	3.5	1.6	1.1	5.2	5.7	7.7	4.5
Min		0.8	0.0	0.0	0.0	3.4	3.9	3.4	2.5
Max		3.4	4.9	3.3	2.8	6.6	7.3	12.8	6.8

⁺, *, **, *** indicates significance at 0.1, 0.05, 0.01, and 0.001 probability levels, respectively.

NMLN = NaCRRI Low Nitrogen; NMON = NaCRRI Optimum Nitrogen; KBMD = Kiboko Managed Drought; KBWW = Kiboko Well-Watered.

‡ Error was used as the F- test. †Mean squares are expressed on the basis of genotype means within environment (2 reps).

The means for grain yields of hybrids followed the same trend across seasons and management environments (Table 11), as was also true in individual testing environments (Table 10). However, this was at a lower level of significance and reduced means. Analysis of all environments combined (Table12) showed that they were different at a highly significant level ($P < 0.001$). Likewise, highly significant variation was revealed among hybrids for grain yield, EPP, and ASI. The range of variation among hybrids was 3.0 to 4.7t/ha for grain yield, 0.8 to 1.1 for EPP, and 0.3 to 6.7 d, for ASI

Table 11: Analysis of variance for grain yield (t/ha) across seasons under stressed and non-stressed environments

Sources of Variation	df	† LN MS [√]	ON MS	MD MS	‡ WW MS	F-test Denominator
Season(S) or Env(E)	1	49.59	3.41	5.88*	257.07**	Rep/S or Rep/E
Rep/S or Rep/E	2	24.54***	11.95***	0.17 NS	2.60*	PE
Hybrids(H)	42	0.52	0.78 +	0.59 +	2.26*	H x S or H x E
GCA	11	0.45	1.12*	1.49*	6.58+	GCA x S or GCA x E
SCA	31	0.54	0.66	0.26	0.73	SCA x S or SCA x E
H x S or H x E	42	0.61 **	0.50 +	0.37*	1.23**	PE
GCA x S or GCA x E	11	0.60*	0.39	0.41*	2.73***	PE
SCA x S or SCA x E	31	0.62**	0.54 +	0.36*	0.70	PE
Pooled Error (PE)	62	0.29	0.34	0.20	0.53	
CV		0.28	0.15	0.45	0.17	
Mean		2.7	5.4	1.4	6.2	
Min		1.1	4.0	0.2	3.9	
Max		3.7	6.5	2.6	7.9	

+, *, **, *** indicates significance at 0.1, 0.05, 0.01, and 0.001 probability levels, respectively.
† LN = Low Nitrogen; ON = Optimum Nitrogen; MD = Managed Drought; WW = Well Watered
‡ WW = two different locations in different seasons.[√]Mean squares are expressed on the basis of genotype means across season or environment (2 reps).

Table 12: Combined analysis of variance and means for grain yield (t/ha) and agronomic traits across all environments and seasons

Sources of Variation		df	GY† t ha ⁻¹ MS [√]	EPP no MS	ASI d MS	F-test Denominator
Env(E)		4	390.14***	3.77***	605.57***	PE
Env. Season(E.S)		3	19.63	0.08	101.77***	Rep/E
Rep/E		8	9.81***	0.07	5.29***	PE
Hybrids(H)		42	1.36***	0.04***	16.58***	H x E x S
	GCA	11	3.39***	0.11***	51.29***	GCA x E x S
	SCA	31	0.64	0.01	4.26	SCA x E x S
H x E		168	1.07***	0.01	4.16*	H x E x S
	GCA x E	44	2.25***	0.023	10.03***	GCA x E x S
	SCA x E	124	0.56	0.01	2.08	SCA x E x S
H x E.S		126	0.50**	0.02	2.91***	PE
	GCA x E x S	33	0.47+	0.02	2.35*	PE
	SCA x E x S	93	0.51**	0.01	3.10***	PE
Pooled Error		297-365	0.33	0.05	1.48	
Cv			0.21	0.37	0.59	
Mean			3.9	0.9	2.9	
Min			3.0	0.8	0.3	
Max			4.7	1.1	6.7	

+, *, **, *** indicates significance at 0.1, 0.05, 0.01, and 0.001 probability levels, respectively;

‡ AD, anthesis date; ASI, anthesis silking interval; EPP, ears per plant; GY, grain yield.

[√]Mean squares are expressed on the basis of genotype means across environments and seasons (2 reps).

4.1.1 Mean squares of general and specific combining abilities in individual environments and across environments

Greater mean square of GCA compared to SCA for grain yield were observed in all environments (Table 10) with exception of low-N environment at NaCRRI during the 2009B season (NMLN 09B). Across seasons (Table 11), GCA effects were significant in all management environments except for low-N stress (LN), while SCA was not significant in any of the management environments. Interactions of GCA and SCA with

seasons within management environments were significant in several cases. Across the combined environments (Table12), GCA was significant while SCA was not. Higher mean squares were observed for GCA than for SCA for grain yield, EPP and ASI. Interactions of GCA and SCA with managed environments and seasons within managed environments were significant for grain yield, but the magnitude was lower than were GCA and SCA mean squares. It is clear that hybrids performed differently in the different managed environments, and that the relative performance of specific hybrids depended on both the managed environment and the specific season.

4.1.2 Components of variance for GCA and SCA in individual environments and across environments

To determine the relative importance of GCA and SCA, the ratios of GCA components of variance were compared to that of total genetic variance (Baker's ratio) and total phenotypic variance (NS-CGD) (Table13, Figures 1-2).

In general, additive genetic variance was more important than non-additive effects for most traits under most environments tested. For grain yield, Baker's ratio showed that GCA accounted for 66% of the total genetic variance across optimum N (ON) environments, 97% across well watered environments (WW), 100%, across managed drought environments (MD), and 87% for the combined environments. This indicated that additive effects were more important than non-additive effects for grain yield in these environments. Ratios of 100% occurred in cases in which the variance component estimate for SCA was 0 or negative.

Table 13: Means, variance components for General and Specific Combining ability, Baker's ratio and Narrow Sense Coefficient of Genetic Determination for grain yield EPP, ASI, and Lsene at each environment and across environments

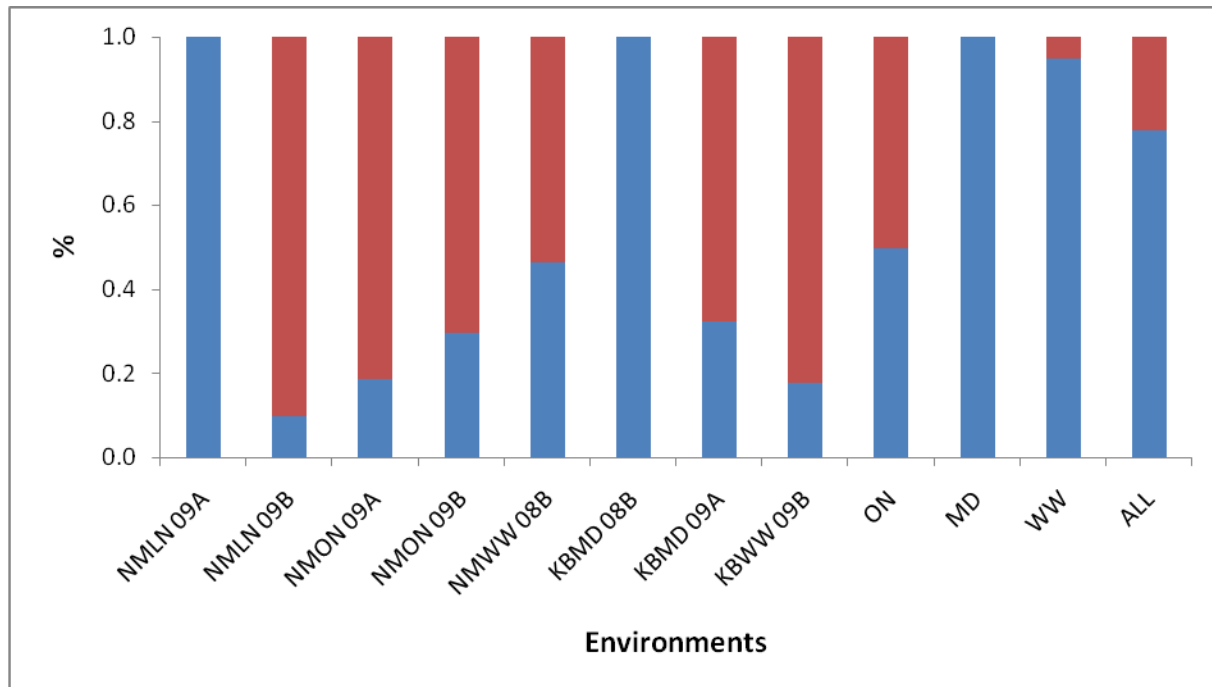
	†LN9A	LN9B	ON9A	ON9B	MD8B	MD9A	KBWW9B	NMWW8B	Across				
									LN	ON	MD	‡WW	†ALL
Means													
GY	1.9	3.5	5.2	5.7	1.6	1.1	7.7	4.5	2.7	5.4	1.4	6.2	3.9
EPP	0.95	0.96	1.08	1.15	0.53	0.62	1.12	1.01	0.95	1.12	0.58	1.05	0.93
ASI	2.24	3.17	0.78	0.39	5.99	9.00	0.76	0.86	2.69	0.67	7.28	0.91	2.89
Lsene	1.67	2.48				3.83			2.06				
GCA													
GY	0.02	0.05	0.05	0.07	0.18	0.09	0.58	0.13	0.00	0.06	0.08	0.30	0.06
EPP	0.000	0.001	0.006	0.003	0.008	0.001	0.005	0.000	0.000	0.004	0.002	0.002	0.001
ASI	0.30	0.44	0.34	0.14	5.22	5.60	0.12	0.68	0.49	0.07	5.22	0.35	0.94
Lsene	0.17	0.11				0.05			0.12				0.06
SCA													
GY	0.00	0.50	0.22	0.18	0.00	0.18	2.71	0.15	0.00	0.06	0.00	0.02	0.02
EPP	0.000	0.005	0.000	0.002	0.000	0.000	0.021	0.003	0.000	0.002	0.000	0.000	0.000
ASI	0.02	0.82	0.24	0.00	4.43	5.08	0.50	0.68	0.02	0.19	0.31	0.00	0.14
Lsene	0.20	0.09				0.09			0.00				
Baker's ratio													
GY	1.00	0.18	0.31	0.45	1.00	0.49	0.30	0.63		0.66	1.00	0.97	0.87
EPP		0.30	1.00	0.73	1.00	1.00	0.32	0.11		0.79	1.00	1.00	0.99
ASI	0.97	0.52	0.74	1.00	0.70	0.69	0.32	0.67	0.98	0.42	0.97	1.00	0.93
Lsene	0.61	0.71				0.52			0.05				1.00
NS-CGD													
GY	0.15	0.11	0.15	0.23	0.64	0.31	0.25	0.41	0.00	0.15	0.28	0.30	0.17
EPP	0.00	0.12	0.56	0.35	0.54	0.05	0.25	0.04	0.02	0.04	0.21	0.27	0.06
ASI	0.49	0.24	0.48	0.18	0.70	0.50	0.20	0.46	0.39	0.09	0.58	0.29	0.35
Lsene	0.53	0.40				0.30			0.39				0.26

NMLN = NaCRRRI Low Nitrogen; NMON = NaCRRRI Optimum Nitrogen; NMWW = NaCRRRI Well-Watered; KBMD = Kiboko Managed Drought; KBWW = Kiboko well-watered; LN = Low-N; ON = Optimum N; MD = Managed Drought; WW = Well-Watered; All = All environments combined.

EPP = number of ears per plant; ASI = anthesis silking interval; Lsene = leaf senescence; GY = grain yield. NS-CGD = Narrow Sense Coefficient of Genetic Determination. ‡WW = two different locations in different seasons. †ALL = Lsene comprises two seasons under LN and one season under MD

The narrow sense coefficient of genetic determination (NS-CGD) was calculated to assess the degree of heritability of performance (Table 13). In general, high values of the coefficient were observed for anthesis-silking interval and leaf senescence, implying that it should be easy to select for improvement in these traits, which might indirectly increase yield. For grain yield, this coefficient varied from 0% in LN to 30% under well-watered environments (WW). In individual seasons, the highest coefficient of NS-CGD (64%) for grain yield was observed at KBMD08B while the smaller coefficient (11%) was at NMLN 09B.

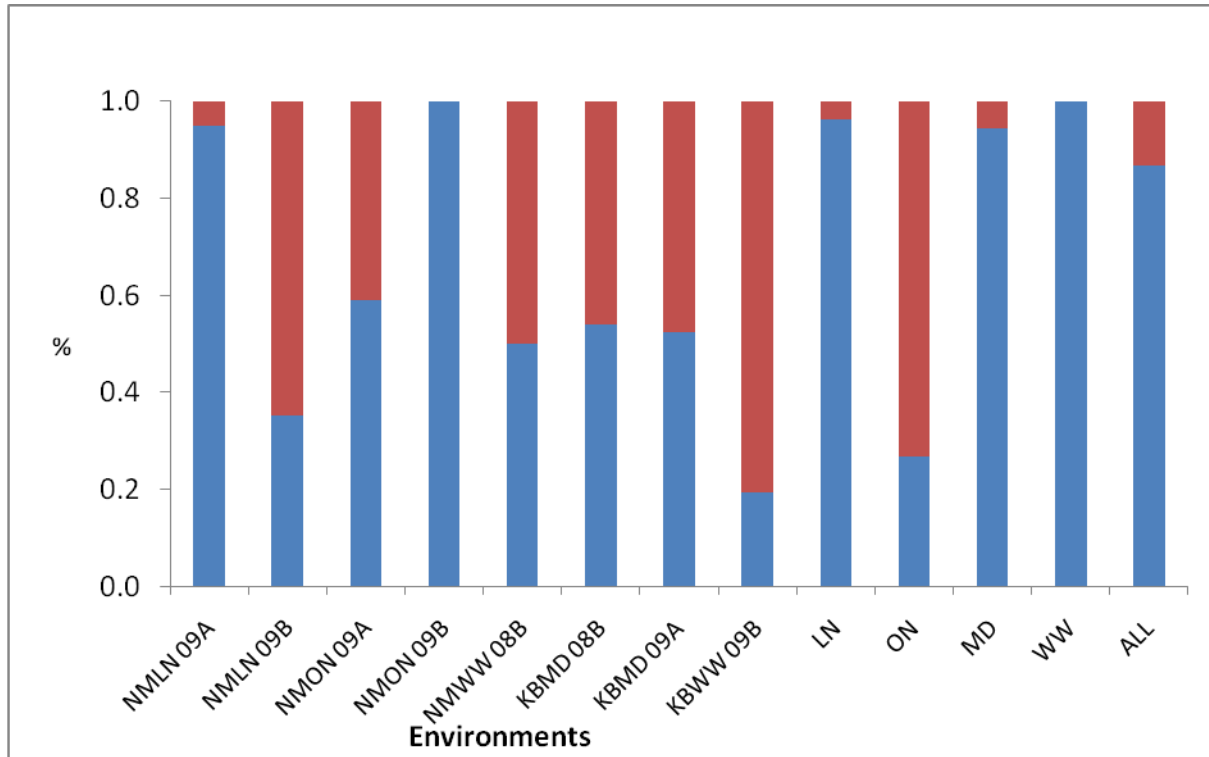
Figure 1: Baker's ratio of GCA (blue bar) and SCA (red bar) variance components for grain yield within and across environments



NMLN= NaCRRI Low Nitrogen= NMON= NaCRRI Optimum Nitrogen; NMWW= NaCRRI Well-Watered; KBMD=, Kiboko Managed Drought; KBWW=, Kiboko Well-Watered; LN= Low-N; ON= optimum N; MD= Managed Drought; WW= Well-Watered; All = All environments combined.

Conversely, in individual environments, the smallest amount of additive genetic variance for EPP was observed under well watered condition at NaCRRI in 2008B (11%) and for ASI at Kiboko in 2009B (32%).

Figure 2: Ratio of GCA (blue bar) and SCA (red bar) genetic variance for anthesis-silking interval within and across environments



NMLN= NaCRRRI Low Nitrogen= NMON= NaCRRRI Optimum Nitrogen; NMWW= NaCRRRI Well-Watered; KBMD=, Kiboko Managed Drought; KBWW=, Kiboko Well-Watered; LN= Low-N; ON= optimum N; MD= Managed Drought; WW= Well-Watered; All = All environments combined.

4.1.3 Evaluation of G x E magnitude across stressed and non stressed environments

Previous analyses revealed significant GCA x E, SCA x E and G x E interactions (Tables 11 and 12). To assess the magnitude of G x E, the ratio of various components contributing to the total phenotypic variance was determined (Table 14) following the formula of Table 9. Across season analysis for managed drought stress (MD), results

revealed that the hybrid contribution (27%) was higher than the hybrid by season (H x S) interaction (21%) but lower than the error variance (52%).

Similarly, across well-watered management environments (Table 14), the hybrid contribution to the phenotypic variance was higher (37%) than it was in the drought environment (27 %) and higher than H x E (25%), but lower than the error variance (38%). Under low-N stress environments (LN), the variance component for hybrids was absent. However, there was a large contribution from hybrid x season (36%), indicating the performance varied according to season. In contrast, under optimum N (ON) environments, differences among hybrids were observed and contributed 25% to phenotypic variance. However, the high error (61%) reduced the percentage contribution of hybrids (Table 14).

Under stress (combined analysis of drought and low-N), there was low variation among hybrids (3% of phenotypic variance) and a higher interaction between hybrid, environment and season (46%) than the interaction in the non-stressed environments (combined analysis of HN and WW, 36%). Across non-stressed environments, there was also large interaction of hybrids with environment and season (H x E x S), in addition to a large error variance (35%).

Table 14: Ratio of different components of variance for yield compared to phenotypic variance

Components	Environment	†Vp (%)	Components	Environments	†Vp (%)
Across WW			Across ON		
Hybrids (H)	0.52	37	Hybrids (H)	0.14	25
H x E	0.35	25	H x S	0.08	14
PE	0.53	38	PE	0.34	61
V(Ph)	1.40	100	V(Ph)	0.56	100
Across MD			Across L N		
Hybrids (H)	0.11	27	Hybrids (H)	-0.05	0
H x S	0.08	21	H x S	0.16	36
PE	0.20	52	PE	0.29	64
V(Ph)	0.39	100	V(Ph)	0.45	100
Across W level			Across N level		
Hybrids (H)	0.18	18	Hybrids (H)	-0.06	0
H x WM	-5.84	0	H x S	0.23	30
H x S/WM‡	0.46	46	H x E	0.21	28
PE	0.35	35	H x S x E	0.02	3
V(Ph)	0.98	100	PE	0.31	40
			V(Ph)	0.78	100
Stress			Non Stress		
Hybrids (H)	0.01	3	Hybrids (H)	0.30	24
H x SM	0.03	6	H x OM	0.06	5
H x S/SM	0.25	46	H x OM x S	0.44	36
PE	0.24	45	PE	0.43	35
V(Ph)	0.54	100	V(Ph)	1.22	100
All					
Hybrids (H)	0.22	22			
H x E	0.26	26			
H x S/E	0.18	18			
PE	0.33	34			
V(Ph)	0.98	100			

H = Hybrid; E= Environment; S = Season; MS = Mean Square; PE = Pooled Error

†Vph= phenotypic variance; WW= Well-Watered; WM= Water Management; LN= Low Nitrogen; ON= Optimum Nitrogen; OM= Optimum management (non-stressed); MD= Managed Drought; SM= Stress Management; all = all environments combined.

‡The WW management environment was planted at two locations, in different seasons, so in all analyses involving the WW environment, seasons were analyzed as a nested effect within environment.

When performance of hybrids was compared under different watering regimes (drought stress vs. well watered) (Table 14), results showed a higher contribution (46 %) of the interaction of hybrids by watering regime and season than of the hybrid contribution alone (18%). In contrast, under different N levels, there was no hybrid contribution to total phenotypic variance (0%). The interactions of hybrids with season and with environment contributed 30 % and 28% of the phenotypic variance, respectively.

Under combined environments (Table 14), hybrids accounted for 22% of the total phenotypic variance, while their interaction with environment contributed 26%, and 18% with season within environment, error variance contributed 34%. Therefore, despite substantial interactions of hybrids with environments and seasons, meaningful differences were still observed among hybrid means when averaged across all the eight testing environments.

4.1.4 General and specific combining ability effects for grain yield

Significant GCA effects were observed for grain yield for at least one parent in each of the management environments except for low-N (Table 15). The highest GCA effects for grain yield (for combined environments) were observed for inbred line CML379 (0.33 t/ha), followed by CZL 02004 (0.22 t/ha) and CZL00003 (0.20 t/ha). The lowest value for GCA effect was observed in inbred line CZL 02008 (-0.51 t/ha).

Of the three best performing inbreds, CZL 02004 yielded consistently well across all environments, while CML 379 showed a negative GCA effect (-0.12 t/ha) when averaged

across the two seasons of optimum N and CZL 00003 performed poorly under both low-N (-0.32 t/ha) and drought stressed (-0.32 t/ha) environments. In contrast, inbred CZL 02008 performed poorly across all environments. The GCA effects of other inbreds varied considerably across environments.

Table 15: General combining ability effects (GCA) of twelve maize inbred lines for grain yield (t/ha) within and across environments

	NMLN 09A	NMLN 09B	NMON 09A	NMON 09B	KBMD 08B	KBMD 09A	KBWW 09B	NMWW 08B	Across				
									LN	ON	MD	WW	All
CZL 0003	-0.17	-0.55**	0.38	0.27	-0.25+	-0.34*	-0.30	-0.02	-0.32	0.44***	-0.32+	1.00*	0.20*
CZL 00034	0.32	-0.14	0.26	0.04	-0.72***	-0.44**	0.90**	0.29*	0.30	0.14	-0.62*	0.22	0.01
CZL 02004	-0.06	0.44*	0.24	0.35*	0.55***	0.21	-0.89*	0.05	0.05	0.21+	0.30+	0.31	0.22*
CZL 02008	-0.25	-0.20	-0.15	-0.35+	-0.08	-0.3+	-0.88*	-0.68***	-0.11	-0.35*	-0.19	-1.41**	-0.51***
CZL 02009	-0.25	0.02	-0.82***	-0.53**	0.15	-0.24	1.55***	-0.63***	0.05	-0.63***	-0.09	-1.12*	-0.45***
CZL 01001	0.11	0.17	-0.28	-0.15	-0.41**	0.17	0.18	-0.30*	0.13	-0.19+	-0.05	-0.79*	-0.22**
CZL 03002	-0.06	0.29	0.10	0.07	0.25	0.24	0.10	-0.29+	0.08	0.16	0.19	0.21	0.16+
CML 499	-0.28	0.18	-0.14	0.24	0.19	-0.20	0.17	0.31+	0.19	0.11	-0.27	-0.47	-0.11
CML 258	0.21	-0.41	-0.15	-0.17	-0.62***	-0.42*	-0.88*	0.33*	-0.27	-0.42*	-0.52*	0.18	-0.26*
CML 341	-0.05	0.28	0.26	0.27	0.13	0.18	0.74*	0.56**	0.01	0.25+	-0.10	0.27	0.11
CML 78	-0.02	-0.18	-0.04	0.32+	0.68***	0.25+	-1.05**	-0.07	-0.15	0.14	0.44*	0.19	0.15+
CML 379	0.43	-0.11	0.23	-0.58**	0.23+	0.61***	0.17	0.43**	0.13	-0.12	0.43*	0.87*	0.33***
Mean(t/ha)	1.9	3.5	5.2	5.7	1.6	1.1	7.7	4.5	2.7	5.4	1.4	6.2	3.9
SE‡	0.17	0.19	0.20	0.19	0.15	0.15	0.31	0.15	0.21	0.12	0.18	0.45	0.10

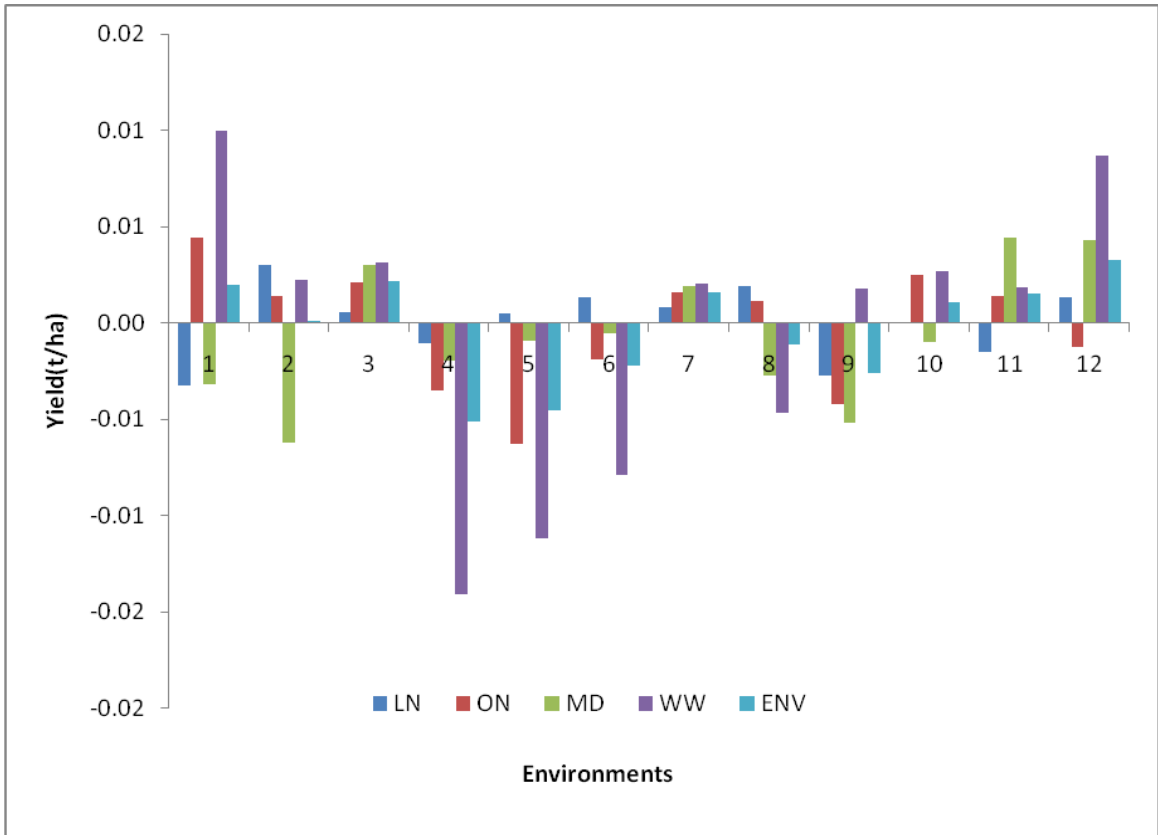
⁺, ^{*}, ^{**}, ^{***} indicates significance at 0.1, 0.05, 0.01, and 0.001 probability levels, respectively.

NMLN= NaCRRI Low Nitrogen; NMON= NaCRRI Optimum Nitrogen; NMWW= NaCRRI Well-Watered; KBMD = Kiboko Managed Drought; KBWW = Kiboko Well-Watered; LN= low-N; ON; optimum N; MD= managed drought; WW= Well-Watered; All = All environments combined.

‡ Standard error of the mean.

Under managed drought, inbreds CML 379 and CML 78 gave the best GCA values (0.43 and 0.44 t/ha, respectively) while CZL 00034 and CML 258 gave the worst GCA values (-0.62 and -0.52 t/ha, respectively). However, under low-N, inbred line CZL 00034 gave the highest GCA effect (0.30 t/ha). CML 258 performed poorly in most environments. Inbred CZL 00003 showed the highest GCA effect across optimum N and well-watered environments (0.44 t/ha and 1.00 t/ha, respectively). However, this line was among the worst-performing under low-N and drought stress (GCA = -0.32 t/ ha in each). Figure 3 highlights the consistency or inconsistency of GCA effects in different environments.

Figure 3: General combining ability (GCA) effects for grain yield of twelve maize inbred lines in a diallel study evaluated across stress and non-stress environments.



LN= Low-N, ON = Optimum N; MD = Managed Drought; WW = Well-Watered; ENV = Combined environments.
 1= CZL 00003; 2=CZL 00034; 3= CZL 02004; 4= CZL 02008; 5= CZL 02009; 6= CZL 01001; 7= CZL 03002; 8= CML 499; 9= CML 258; 10= CML 341; 11= CML 78; 12= CML 379.

The grain yield performance of the F1 hybrids varied with environments. However, it was observed that the best hybrids over all environments also showed high performance under optimum environments (ON and WW) and conversely, if a cross performed poorly under those environments, its overall performance was poor. Additionally, the top five hybrids for overall mean yield also exhibited higher-than-average in most individual environments (Table 16).

Table 16: Specific combining ability effects (SCA) and corresponding hybrid means (in parenthesis) for 5 SCs with the highest mean yield and 5 with the lowest mean yield (t/ha) across environments.

Crosses	†LN	ON	MD	WW	ACROSS
	0.35	0.42	0.84*	0.39	0.50*
CZL 00003/CML 341	(2.7)	(6.5)	(1.9)	(7.9)	(4.7)
	-0.27	0.82	0.00	0.09	0.16
CZL 03002/CML 379	(2.6)	(6.3)	(2.1)	(7.4)	(4.6)
	0.30	-0.37	0.29	0.61	0.21
CML 78/CML 379	(3.0)	(5.1)	(2.6)	(7.9)	(4.6)
	0.13	-0.14	0.42	0.43	0.21
CZL 00034/CML 379	(3.3)	(5.3)	(1.7)	(7.7)	(4.5)
	0.47	-0.19	-0.16	0.92	0.26
CZL 03002/CML 341	(3.3)	(5.6)	(1.4)	(7.6)	(4.5)
	-0.23	-0.63	-0.17	-0.29	-0.33
CZL 00003/CZL 02009	(2.2)	(4.6)	(0.8)	(5.8)	(3.4)
	0.05	0.27	-0.25	-0.93	-0.22
CZL 02008/CZL 03002	(2.7)	(5.5)	(1.2)	(4.1)	(3.4)
	0.21	-0.52	-0.02	0.10	-0.06
CZL 02008/CZL 01001	(2.9)	(4.4)	(1.2)	(4.1)	(3.1)
	0.01	-0.26	-0.17	-0.42	-0.21
CZL 02009/CZL 01001	(2.9)	(4.3)	(1.1)	(3.9)	(3.1)
	-0.06	-1.08*	-0.18	-0.84	-0.54
CML 499/CML 258	(2.6)	(4.0)	(0.5)	(5.1)	(3.0)
Mean	2.7	5.4	1.4	6.2	3.9
Min	1.1	4.0	0.2	3.9	3.0
Max	3.7	6.5	2.6	7.9	4.7
LSD	NS	1.43	1.23	2.24	0.71

† LN= Low-N, ON = Optimum N; MD= Managed Drought; WW= well-Watered.

* indicates significance at 0.05; NS = non significant

Averaged over environments, the most consistent and best yielding hybrids were obtained for the cross between CZL 00003/CML 341 (4.7 t/ha, SCA = 0.50), followed by the cross between CZL 03002/CML 379 (4.6 t/ha, SCA = 0.16). The parents of these two crosses also exhibited overall positive but inconsistent GCA effects (Table15). The highest performing hybrid also showed consistently positive SCA effects in all environments, with the highest effect recorded under MD (0.84), though the two parents showed

negative GCA effects in that environment (Table 10). The cross CML 78/CML 379 not only performed well overall (4.6 t/ha) but also performed consistently well in stressed environments (LN=3.0 t/ha and MD=2.6 t/ha). Additionally, these two inbreds exhibited consistently positive GCA effects, except when CML 379 was grown under optimum N conditions (-0.12), and CML 78 under low-N (-0.15). Overall, the top five hybrids also showed positive SCA effects (Table 15). Three of these hybrids involved CML 379 as one of the parents, two included CZL 03002 and another two included CML 341.

In contrast, crosses involving CML 499/CML 258 and CZL 02009/CZL 01001 performed poorly in all environments, with average grain yields of 3.0 and 3.1 t/ha, respectively. These crosses performed especially poorly under drought stress.

4.1.5 General combining ability effects for important agronomic traits

Inbred lines CZL 03002, CML 341 and CZL 02004 had the most desirable GCA effects (0.08; 0.07 and 0.06, respectively) for the number of ears per plant across environments and in most individual environments (Table 17). Under managed drought stress, the best performing line was CZL 02004 (0.9), while under low-N environments the best performing line was CZL 00034 (0.6).

Table 17: General combining ability effects (GCA) of twelve maize inbred lines for agronomic traits across environments.

	EPP~ (no)					ASI (d)					Lsene rating 1-10		
	LN†	ON	MD	WW	ALL	LN	ON	MD	WW	All	LN	MD√	All
CZL 00003	0.01	-0.01	-0.06	0.01	-0.01	1.71**	1.01*	4.00***	0.94*	1.92***	0.17	0.47***	0.28*
CZL 00034	0.06+	-0.04	-0.13*	-0.04	-0.04	0.32	0.36	4.92***	0.68	1.57***	-0.59*	-0.33**	-0.42*
CZL 02004	0.00	0.10*	0.09+	0.07+	0.06**	-0.35	-0.88*	-3.38***	-1.08*	-1.43***	0.48*	0.27*	0.36*
CZL 02008	0.01	-0.07+	-0.07	-0.07+	-0.05*	-0.76*	-0.23	-0.33	-0.29	-0.40+	0.20	-0.02	0.17
CZL 02009	0.02	-0.12**	-0.03	-0.06	-0.05*	-0.01	-0.34	0.26	1.02*	0.23	-0.10	-0.06	-0.10
CZL 01001	-0.06*	-0.02	-0.04	-0.05	-0.04*	0.03	0.44	0.49	0.59	0.39*	-0.62**	-0.21+	-0.43**
CZL 03002	0.02	0.10*	0.10+	0.11**	0.08***	0.24	0.06	-1.04+	0.00	-0.18	-0.07	0.30*	0.13
CML 499	0.01	-0.02	-0.07	-0.05	-0.03	-0.19	-0.44	-0.69	-0.25	-0.39	0.79**	-0.16	0.44*
CML 258	0.04	-0.02	-0.03	-0.05	-0.01	0.68+	-0.34	1.73*	0.26	0.58*	0.31	-0.20	0.09
CML 341	-0.01	0.14**	0.05	0.09*	0.07**	-0.12	-0.12	-1.87**	0.16	-0.49*	0.35+	0.25*	0.23
CML 78	0.01	-0.08*	0.04	-0.04	-0.02	-0.52*	-0.09	-1.27*	-0.60+	-0.62***	-0.31+	-0.21+	-0.27*
CML 379	-0.03	0.02	0.05	0.02	0.02	-0.78**	-0.04	-1.09*	-0.91*	-0.70***	0.00	-0.15	-0.16
Mean	1.00	1.10	0.60	1.10	0.90	2.70	0.70	7.30	0.90	2.90	2.10	3.80	3.00
SE‡	0.03	0.36	0.05	0.04	0.02	0.28	0.39	0.55	0.41	0.21	0.18	0.12	0.15

+, *, **, *** indicates significance at 0.1, 0.05, 0.01, and 0.001 probability levels, respectively.

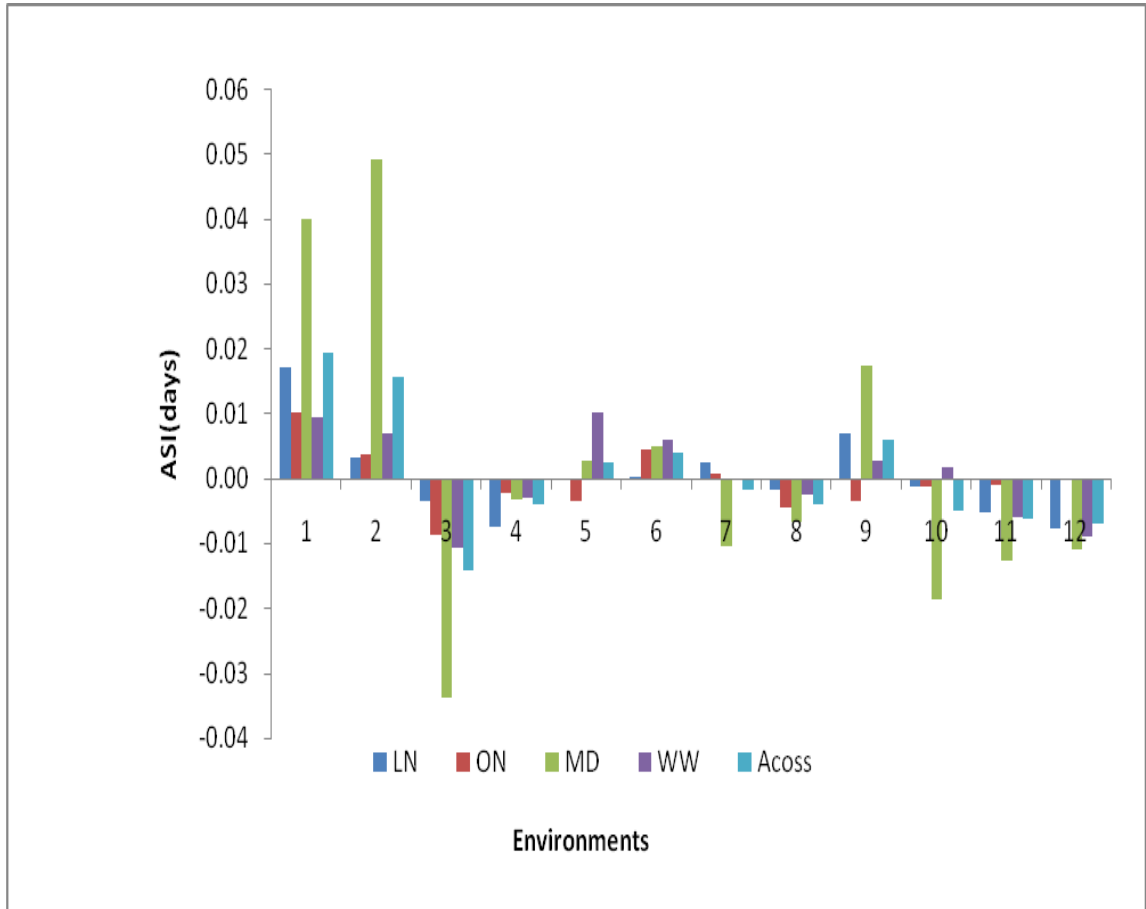
~EPP= number of ears per plant; ASI= anthesis silking interval; Lsene= leaf senescence.

† LN= Low-N, ON = Optimum N; MD= Managed Drought; WW= Well-Watered; All = All environments combined.

‡ Standard error of the mean.

√ Effects based on single season

Figure 4: General combining ability (GCA) effects for anthesis-silking interval of twelve maize inbred lines in a diallel study evaluated across stressed and non-stressed environments.



LN= Low-N; ON; Optimum N= MD; Managed Drought; WW= Well-Watered.
 1= CZL 00003; 2=CZL 00034; 3= CZL 02004; 4= CZL 02008; 5= CZL 02009; 6= CZL 01001;
 7= CZL 03002; 8= CML 499; 9= CML 258; 10= CML 341; 11= CML 78; 12= CML 379.

Estimates of GCA effects for anthesis-silking interval (ASI) were variable among inbreds and between environments (Table 17, Figure 4). Consistently significant and negative GCA values for ASI effects were observed across environments for lines CZL 02004, CZL 02008, CML 379, CML 499, and CML 78. Under managed drought stress, desirable GCA effects were observed in line CZL 02004 (-3.4 d), followed by CML341 (-1.9 d)

and CML 78 (-1.3 d), while under low-N, the worst GCA effects for ASI were CML 379 (-0.78 d), CZL02008 (-0.76 d) and CML 78 (-0.52 d). Averaged across environments, significantly negative GCA effects for leaf senescence were observed for inbreds CZL 01001 (-0.43), CZL 00034 (-0.42) and CML 78 (-0.27) (Table 17), which resulted from significant and negative GCA effects in individual environments. Over all, the inbred lines with negative or small ISA and/or Lsene GCA effects were the ones with higher GCA effects for grain yield.

4.2 Effect of low-N and drought stress on grain yield

As expected, drought and low-N stress caused loss of grain yield in all the genotypes (Table 18), but this loss differed among the genotypes, depending on the season and the type of stress (drought or low nitrogen) (Table 18). The highest yield reduction for an individual genotype was observed under drought stress (97%) rather than low-N stress (80%). Under drought stress, yield reduction ranged from 60% in CZL 02004/CZL02008 to 97% in CZL 00003/CZL 00034, while the average yield reduction for all crosses was 76%. Yield loss for the check was estimated at 71%.

Under low-N stress, grain yield losses ranged from 26% to 80%, with an average loss of 49% (Table 18). Loss in yield of the check was estimated at 51%. Overall, under combined low-N and drought stress, yield losses ranged from 48% to 83%, averaging 64%. The average loss in the check under these conditions was 61%.

Among the 10 genotypes with the greatest losses under drought stress, two of them (CZL 00003/CZL 00034, 97% and CZL 00034/CZL 02004, 87%) were also among the top five

with high losses under low-N stress (67.4% for CZL 00003/CZL 00034 and 58% for CZL 00034/CZL 02004).

Table 18: Relative loss (%) of grain yield (t/ha) under conditions of low nitrogen and drought for the check and for hybrids with the highest and lowest yield losses

CROSSES	GY	GY	% Loss	GY	GY	% Loss	Across		
	†WW	MD		ON	LN		NON STRESS	STRESS	% Loss
CZL 00003/CZL 00034	7.2	0.2	96.8	6.3	2.0	67.4	6.7	1.1	83.0
CZL 0003/CML 258	7.9	0.3	95.8	5.5	2.7	51.5	6.7	1.5	77.7
CML 499/CML 258	5.1	0.5	91.0	4.0	2.6	36.4	4.5	1.5	66.8
CZL 01001/CML 258	6.1	0.7	88.1	5.7	3.3	42.4	5.9	2.0	66.1
CZL 00034/CZL 02004	6.3	0.8	86.9	6.3	2.7	58.1	6.3	1.7	72.4
CML 499/CML 379	7.3	1.0	86.0	5.7	3.2	44.8	6.5	2.1	67.9
CZL 00003/CML 78	7.7	1.1	85.6	5.9	3.0	48.5	6.8	2.1	69.6
CZL 00003/CZL 02009	5.8	0.8	85.4	4.6	2.2	52.3	5.2	1.5	70.8
CZL 00003/CZL 02008	6.1	0.9	84.7	5.2	1.8	65.5	5.6	1.4	75.9
CZL 00034/CML 78	6.8	1.1	83.0	5.5	3.1	42.3	6.1	2.1	64.8
Check	6.9	2.0	70.9	6.5	3.2	50.8	6.7	2.6	61.2
CZL 02004/CML 78	6.4	2.0	69.3	5.3	2.1	60.1	5.8	2.0	65.1
CZL 02009/CML 379	5.9	1.9	68.2	4.5	2.8	36.9	5.2	2.3	54.7
CZL 01001/CML 379	6.0	2.0	67.6	5.4	2.3	58.0	5.7	2.1	63.1
CML 78/CML 379	7.9	2.6	67.0	5.1	3.0	41.1	6.5	2.8	56.9
CZL 02009/CZL 03002	5.3	1.8	66.3	5.1	2.2	57.2	5.2	2.0	61.9
CZL 02009/CML78	5.5	1.9	64.9	5.1	3.2	38.1	5.3	2.5	52.0
CZL 02004/CML 379	6.5	2.3	64.2	4.9	3.6	26.2	5.7	3.0	47.9
CZL 03002/CM 78	6.4	2.3	63.7	6.2	1.9	69.9	6.3	2.1	66.7
CZL 02004/CML 379	6.5	2.3	64.2	4.9	3.6	26.2	5.7	3.0	47.9
CZL 02004/CZL02008	5.3	2.1	60.2	6.1	2.4	60.7	5.7	2.2	60.5
Mean	6.2	1.4	76.3	5.4	2.7	49.4	5.8	2.1	63.9
Min	3.9	0.2	60.2	4.0	1.1	26.2	4.1	1.1	47.9
Max	7.9	2.6	96.8	6.5	3.7	79.9	7.2	3.0	83.0

†LN= Low-N; ON= Optimum N; MD= Managed Drought; WW= Well-Watered
GY = grain yield

Generally, crosses involving CZL 00003 as one of the parents experienced severe yield reductions, and the lowest yield reduction was observed in crosses involving CZL 02009 or CZL 02004 as a parent.

When environments were combined for analysis of the influence of stress levels, the mean grain yields were 2.1 t/ha under stressed and 5.8 t/ha under non-stressed environments, indicating over 64% yield loss due to stress. Therefore, a large part of the yield potential was not realized in many genotypes when evaluated under stressed environments. In addition, some genotypes that yielded best without any deficiency in their environments (e.g., CZL 00003/CZL 00034, 6.7 t/ha) had the lowest yield under stressed conditions (1.1 t/ha). The fractional yield reduction was generally greater for genotypes that yielded well in optimum environments than for genotypes that yielded poorly in optimum environments.

4.3 Relationship between grain yield and secondary traits

To determine the relationship between grain yield and secondary traits, Pearson coefficients of correlation were calculated (Table 19). In the drought-stressed environment, a highly significant negative correlation was observed between grain yield and anthesis-silking interval (-0.67^{**} , $p < 0.01$). A positive correlation was observed between grain yield and the number of ears per plant (0.80^{**} , $p < 0.01$, Table 19). No significant correlations were found among other traits in this environment except between the anthesis-silking interval and the number of ears per plant (Table 19)

Table 19: Pearson's correlation coefficients (r) between grain yield and secondary traits across stressed and non- stressed environments

Environment	Traits	GY [†]	EPP	ASI	Lsene
Drought stress	GY	1.00			
	EPP	0.80**	1.00		
	ASI	-0.67**	-0.62**	1.00	
	Lsene	0.07	-0.03	0.27+	1.00
Low-N stress	GY	1.00			
	EPP	0.01	1.00		
	ASI	-0.09	-0.10	1.00	
	Lsene	-0.13	-0.04	0.24	1.00
	Lyelw	-0.36*	-0.07	0.32*	0.79**
Across stress	GY	1.00			
	EPP	0.54**	1.00		
	ASI	-0.57**	-0.47**	1.00	
	Lsene	-0.25	-0.05	0.07	1.00
Across non-stress	GY	1.00			
	EPP	0.39**	1.00		
	ASI	0.00	-0.25	1.00	
Across all	GY	1.00			
	EPP	0.58**	1.00		
	ASI	-0.13	-0.41**	1.00	

⁺,*, ** indicates significance at 0.1, 0.05, and 0.01 probability levels, respectively.

[†]EPP = number of ears per plant; ASI= anthesis-silking interval; GY = Grain yield; Lsene= leaf senescence.

Under low-N stress, significant correlations were observed between grain yield and Lyelw (-0.36*), ASI and Lyelw (0.32*) and Lyelw and Lsene (0.79**).

When drought and low-N stressed management environments were combined, grain yield was significantly correlated to EPP (0.54**) and to ASI (-0.57**). No significant correlation was observed between grain yield and Lsene (-0.25), and EPP was negatively correlated to ASI (-0.47**, Table 19). In contrast, under non-stressed environments (well watered and optimum N) (Table 19), significant correlation was observed only between grain yield and EPP (0.39**). When all environments were combined (Table 19),

significant correlations were found between yield and EPP (0.58**), and between EPP and ASI (-0.41**). Correlations among all other traits were low and non-significant.

4.4 Analysis of G x E interaction by AMMI and GGE

The evaluation of G x E interaction for grain yield was extended to the Additive Main effect and Multiplicative Interaction (AMMI) analysis (Table 20). The highly significant interaction was evaluated using the first two Interaction Principal Component Axes (IPCA), IPCA1 and IPCA2. Both of these were significant and together accounted for 62% (46% + 16%) of the sum of squares for interaction. The residual SS was non-significant, indicating that the model effectively partitioned sum of squares for treatments.

An AMMI biplot was used to visualize the relationship between environment and genotypes (Figure 5). It is a two dimensional graph displaying the mean yield on the abscissa and the first axis IPCA scores on the ordinate, with each genotype and each environment shown as separate points.

Table 20: AMMI analysis of variance for maize grain yield of 43 hybrids across 8 environments

Source	df	SS	MS
Total	703	4381	6.23
Treatments (Genotypes and Environment combinations)	351	3924	11.18***
Genotypes	43	135	3.13***
Environments	7	3348	478.30***
Block	8	122	15.20***
Interactions	301	442	1.47***
IPCA	49	203	4.14***
IPCA	47	72	1.52*
Residuals	205	167	0.82
Error	344	335	0.97

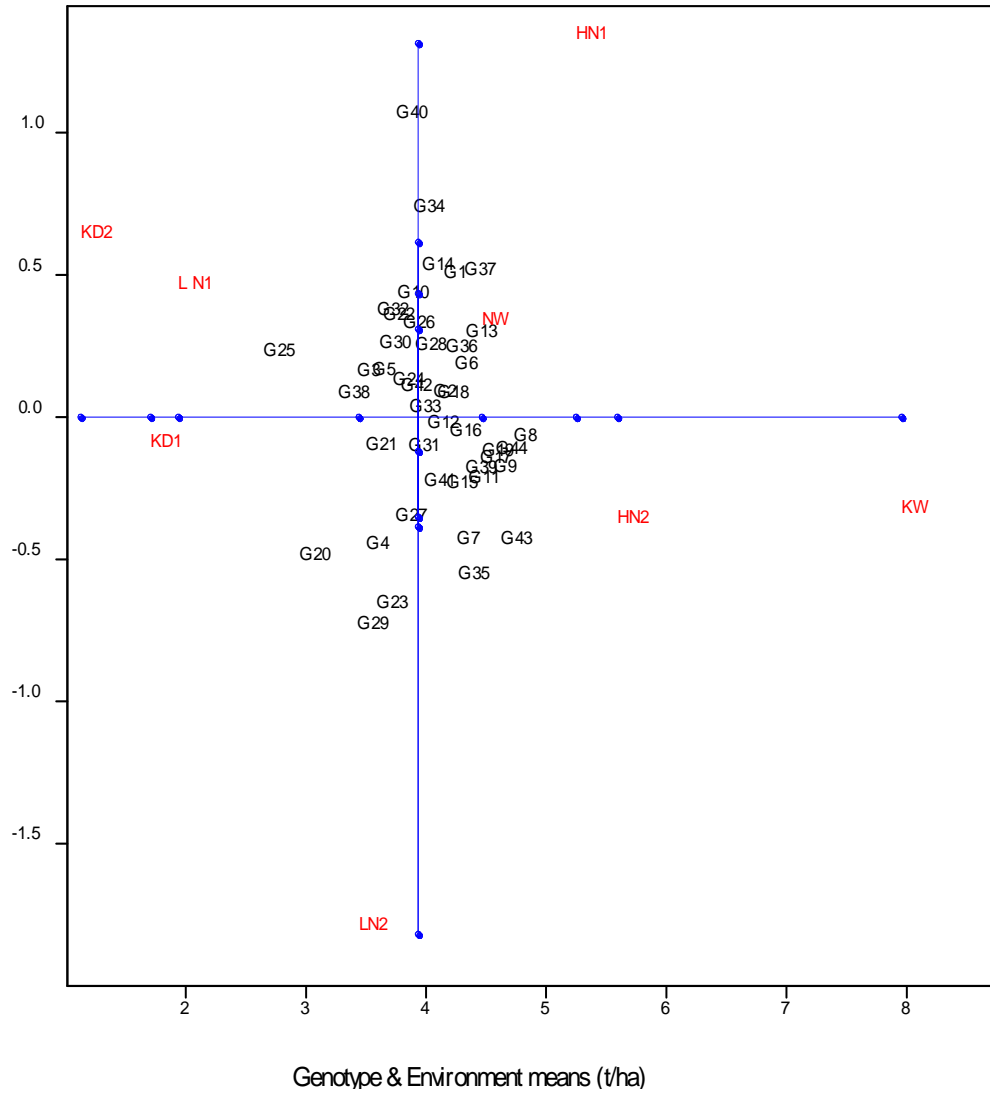
*, **, *** indicates significance at 0.05, 0.01, and 0.001 probability levels, respectively;

On the biplot graph (Figure 5), genotypes and environments that interact favorably have IPCA1 scores with the same sign, and thus appear in the in the same upper quadrant or the lower quadrant on the graph. A larger absolute IPCA1 score indicates that an environment or a genotype has G x E interactions that are larger than those for other environments or genotypes. The lower mean for yield in the stressed environments was shown by their position to the left of the vertical line, with the converse true for the optimal environments. Among the stressed environments, low N season B (LN2) exhibited the highest IPCA1 score, meaning that it was highly interactive. By comparison, kiboko managed drought in the first season (KD1), (low interaction) had the IPCA1 score nearest 0, indicating that genotype differences in that environment closely reflected the genotype differences when averaged over the eight testing environments. In the optimum environments, optimum N season A (HN1) showed the highest interaction, as indicated by its high IPCA1 score.

The well-watered environment at Kiboko well-watered (KW) and Optimum N in season 2009B at NaCRRI (HN2) provided high yielding environments with moderately negative IPCA values as they are located far from the vertical axis and a bit below the horizontal axis. In contrast, the mean for the low- N environment of 2009B at NaCRRI (LN2 = low N in 09B) was slightly below average, and had a strongly negative IPCA score. In contrast, the high N environment; HN1 (optimum N in 09A) was high yielding and had a positive IPCA1 score (highly positively interactive), whereas environment, LN1 (low N in 09A) was lower yielding than LN2, but positively interactive with genotypic IPCA1 scores (positive IPCA1 score). Similar results were obtained for KD2 (managed drought in 09A). Moderately high yield and positive interaction (positive IPCA1 score) were observed at NW (NaCRRI, well-watered).

Overall, the IPCA1 scores for environments showed no clear pattern related to location, season, or the presence or type of stress.

Figure 5: AMMI biplot of 43 hybrids evaluated across 8 environments



LN1= Low-N 09A; LN2= Low-N 09B; HN1= Optimum N 09A; HN2= Optimum N 09B; MD1= Managed Drought 08B; MD2= Managed Drought 09A; KW= Kiboko Well-Watered; NW= NaCRRI Well-Watered.

The AMMI analysis (Figure 5) showed that hybrids (G43 (CML 78 x CML 379), G8 (CZL 00003 x CML 341), G44 (check), G9 (CZL 00003 x CML 78), G11 (CZL 00034 x CZL 03002), G35 (CZL 03002 and G7 (CZL 00003 x CML 258) were high yielding, as indicated by their position beyond the mean grain yield on the horizontal axis. However, all these except G8 (CZL 00003 x CML 341) had negative IPCA scores, indicating that

they interacted negatively with environments that had a positive IPCA, as shown by their IPCA1 scores having opposite signs.

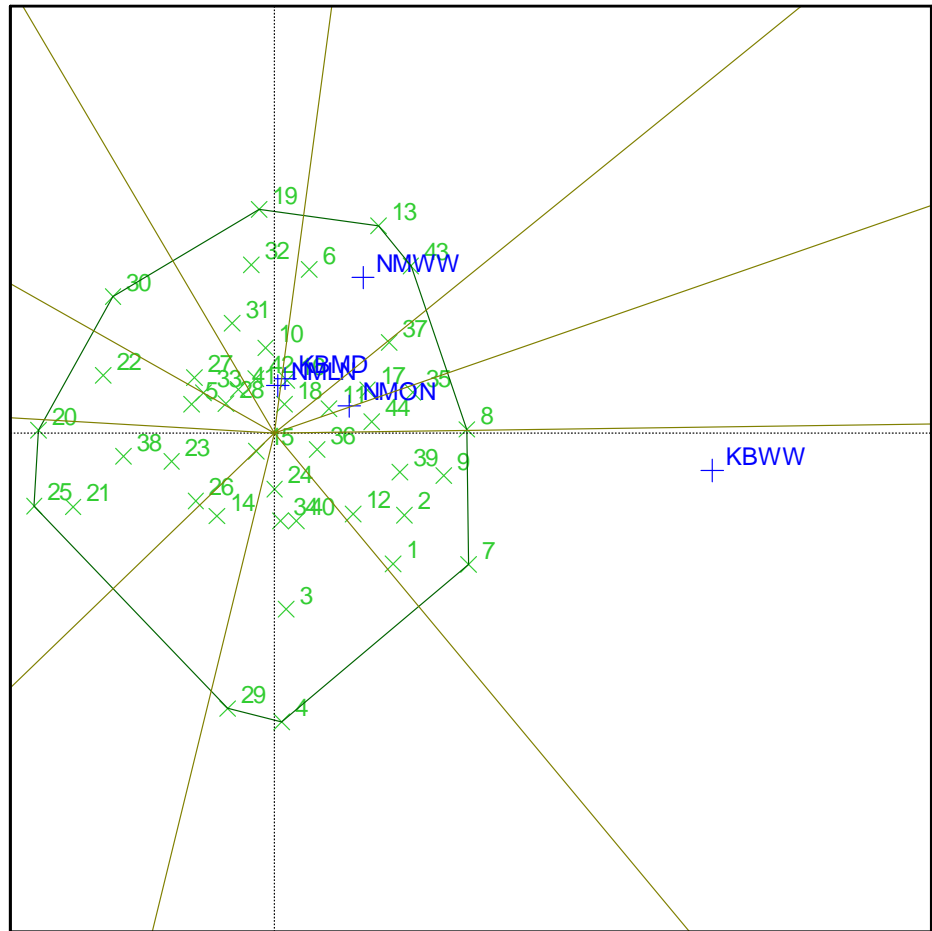
Several hybrids, G13 (CZL 00034 x CML 379), G36 (CZL 03002 x CML 78), G6 (CZL 00003 x CML 499), and G37 (CZL 03002 x CML 379), yielded well and interacted positively with the well-watered environment at NaCRRI (NW), as indicated by their position in the upper-right quadrant of the graph and their distance from the intersection of the mean yield with 0 IPCA (The intersection is the origin of the graph.). In addition, genotypes G8 (CZL 00003 x CML 341), G16 (CZL 02004 x CZL 0001), G33 (CZL 01001 x CML 78), and G12 (CZL 00034 x CML 78), yielded above the mean and were stable, as seen by their proximity to the horizontal axis. The most high yielding and stable hybrids were G8, G16, and G12.

To better understand the magnitude of G x E across stressed and non-stressed environments for grain yield, the GGE analysis was used, with five management environments -- (2 seasons each averaged for NMLN, NMON, and KBMD, with only one season each from NMWW and KBWW). Environments or genotypes near the origin of the graph show little G x E interaction, while those far from the origin indicate large G x E effects. Genotypes enclosed within the same sector as a particular environment are those that interacted positively with that environment. In any sector that includes a test environment, genotypes at the perimeter angle of the polygon were the “winners” (the best performing in that environment).

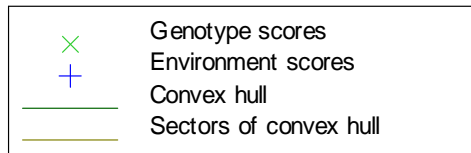
GGE analysis involving the two IPCA axes explained 81% of the yield variation due to GGE, with IPCA1 accounting for 70%. Three groups of mega-environments were identified (Figure 6): KBWW, NMON, and the combination of NMWW, NMLN and KBMD. In these mega-environments, some of the hybrids were located at or near the origin, indicating little interaction (stable genotypes) between these hybrids and environments. These were G15 (CZL 02004 x CZL 02009), G8 (CZL 00003 x CML 341), G36 (CZL 03002 x CML 78), and G 24(CZL 02008 x CML 78). Others, such as G13 (CZL 00034 x CML 379), G43 (CML 78 x CML 379, G37 (CZL 03002 x CML 379), G35 (CZL 03002 x CML 341), G9 (CZL 00003 x CML 78) and G7 (CZL 00003 x CML 258) were found far from the origin, indicating a significant interaction with environments (least stable genotypes)

Figure 6: The winning genotypes in each mega-environment (group of environments) for grain yield.

Scatter plot (Total - 81.10%)



PC1 - 70.43%



NMLN = NaCRRRI Low Nitrogen
 NMON = NaCRRRI Optimum Nitrogen
 NMWW = NaCRRRI well-Watered
 KBMD = Kiboko Managed Drought
 KBWW = Kiboko well-Watered

The best three hybrids in the first mega-environment (KBWW) were G7 (CZL 00003 x CML 258), G8 (CZL 00003 x CML 341) and G9 (CZL 00003 x CML 78), as shown by their position within the same sector as KBWW and their large distance from the origin. Several other hybrids, including G1 (CZL 00003 x CZL 00034), G2 (CZL 00003 x CZL 02004), G12 (CZL 00034 x CML 78), G39 (CML 499 x CML 379) G44 (check) and G36 (CZL 03002 x CML 78), were also within this sector at some distance from the origin, showing that many genotypes were better adapted in this environment than the same genotypes were in other environments. G8 (CZL 00003 x CML 341) was the highest yielding stable genotype, as it had a higher mean and was located on the horizontal axis corresponding to no interaction with environment. In the second mega-environment (NMON), the best hybrids were G35 (CZL 03002 x CML 341), G37 (CZL 03002 x CML 379) and G17 (CZL 02004 x CZL 03002). In the third mega-environment (NMHN, NMLN, KBMD), NMWW was highly interactive and had a higher mean than did NMLN and KBMD. In this mega-environment, the best hybrids were G43 (CML 78 x CML 379) and G13 (CZL 00034 x CML 379), followed by G37 (CZL 03002 x CML 379) and G6 (CZL 00003 x CML 499).

CHAPTER FIVE

DISCUSSION

Analyzed across management environments or seasons, GCA effects were significant for grain yield, EPP and ASI, but SCA effects were not, suggesting that additive gene action was more important than non-additive for these traits (Musila *et al.*, 2010). However, GCA and SCA effects associated with grain yield and ASI for the parental inbred lines and hybrids varied with the environment (Tables 11 & 12), indicating that the relative performance of hybrids in this study depended on both the management environment and the specific season in which they were tested.

Components of phenotypic variance for grain yield were relatively high for error variance (45%) and for G x E interactions (52%) under the combined stress environments (of low-N and drought). Under this management environment, the hybrid contribution to total phenotypic variance was very low (3%), indicating that hybrid performance was not consistent across the different stress environments. The high coefficients of variation observed for yield under stressed environments (Table 10-11) are possibly the result of the wide variation in ears per plant and kernels per plant. However, under low-N stress, the high CV's may also be partly be due to lower than expected rainfall during the first season of this study, whereas under drought stress, this may be partly explained by elephant damage that occurred in 2009A which affected some plots of the trial resulting in low yields and high variability. In addition, under low-N stress termite damage might have affected the number of ears per plant, and may partly explain the lack of differences

in grain yield observed under low-N stress (Table 11 & 16). Reducing the error variance and reducing the effect of G x E interactions will require efforts to obtain greater experimental uniformity, more replications and more seasons of testing. Similar findings were reported by Bänziger *et al.* (1997) under low-N stress conditions. Conversely, Presterl *et al.*, (2003) working on European flint and dent maize found no differences between heritabilities at low or optimum N, although genotype by environment interaction and error effects were higher under low-N. High error variability and G x E variation results in low heritability estimates (Richards *et al.*, 2006).

Nonetheless, it was still possible to identify hybrids that performed well across both drought and low-N stress (Tables 16 and 18), as Beck *et al.* (1997) and Bänziger *et al.* (1999) reported. These authors noted that selection under drought stress improves partitioning of either carbohydrates or N compounds to the growing ear, and often results in decreased ear abortion. Yields of four lowland tropical maize populations have been shown to increase when selected for tolerance to mid-season drought under a wide range of N levels (Bänziger *et al.*, 1999).

GCA effects, and thus presumably additive genetic effects, for grain yield were more important than SCA under managed drought stress and optimum environments. However, this observation may not hold true under other managed drought stress since it is based on computation from low GCA component and negative SCA values. Also, it was not possible to compute additive effects under low-N conditions due to negative components for both GCA and SCA. Nevertheless, additive effects appear to be more important for

ASI in environments of drought and low-N stress than in optimum N and well-watering environments, where little variation was recorded for ASI.

The large mean square values observed for GCA and SCA in certain environments indicate that the low level of interaction effects may be due to the stability across environments of the underlying genetic effects.

The estimation of narrow-sense coefficient of genetic determination (NSCGD) under conditions of drought stress (28%) was almost equal to that of the well-watered environments (30%). Though the low value for the drought stressed environment resulted from the small variance component for GCA (0.08), the value for Baker's ratio was 1.00, since the variance component for SCA was negative and was therefore considered to be zero. At Kiboko, under managed drought conditions in 2008 B, NSCGD was higher (0.64) than in other stress experiments, with a GCA variance component of 0.18, and Baker's ratio equal to 1.00. The second season at Kiboko (2009 A) gave a lower NSCGD (0.31). Bolaños and Edmeades (1996) reported a broad-sense heritability value of 0.43 on an entry-mean basis for grain yield of the S_1 progeny of tropical maize under severe stress. The corresponding value across environments was 0.59.

The NSCGD estimates obtained in this study could be low enough to slow breeding progress. As mentioned earlier, the reduction of error variance and the use of additional testing to reduce interaction effects could compensate for these somewhat low heritability estimates. It appears that promising genetic gain is possible for ASI and Lsene under drought stress (NSCGD = 0.58 for ASI, 0.30 for Lsene) and under low-N (NSCGD = 0.39 for ASI; 0.39 for Lsene). These results are in agreement with earlier findings (Bänzinger *et al.*, 2000; Bolaños and Edmeades 1996) that indicated that selection for

drought tolerance based on grain yield alone is inefficient. These authors also showed increased selection efficiency through the use of secondary traits of adaptive value, such as ASI and, leaf senescence, which show greater genetic variability under drought conditions than in optimum environments. These secondary traits can be useful in selecting for tolerance to stress, as their heritabilities remained high under stress and their genetic correlation with grain yield was increased (Bolaños and Edmeades, 1996, Bänzinger and Lafitte, 1997, Bänzinger *et al.*, 2000, Edmeades *et al.*, 2006).

Inbred lines CZL 02004 and CZL 03002 exhibited consistently positive GCA effects for grain yield in all environments, while CML 379 was the best across stressed environments (managed drought and low-N). CML 341 and CZL 02004 were the best lines across environments for optimum conditions (well watered and optimum N). Furthermore, desirable (negative) GCA effects for ASI were shown by inbred line CZL 02004 under managed drought stress and across all stressed environments, and by CML 379 under low-N. The GCA effect for leaf senescence indicated that CZL 00034 was the best of all inbreds under managed drought stress, and that CZL 01001 was best of all inbreds under low-N conditions and across stressed environments. The best hybrids for grain yield were CML 78/CML 379 under managed drought stress, CZL 00034/CZL 03002 under low-N stress, and CZL 02004/CML 379 when the two stressed environments were combined.

Yield reductions due to stress varied for the different hybrids. On average, managed drought stress environments affected grain yield more (76% reduction) than did low-N stress environments (50% reduction). However, both drought and low-N stress reduced

grain yield of the genotypes by at least 48 %. Under drought stress, CML 78/CML 379, CZL 03002/CML 78, CZL 02004/CML 379 and CZL 01001/CML 78 produced the highest yields with the lowest reductions. Furthermore, hybrids CZL 00034/CZL 03002, CZL 01001/CZL 03002 and CZL 02004/CML 379 performed well compared to other hybrids, especially in the low-N stress environment. Not many hybrids combined lower-than-average reductions from stress with higher-than-average yields in both low-N and drought stressed conditions -- CZL 02004/CML 379, CZL 02009/CML78, and CZL 02009/CML 379. Inbred lines CML 379 and CML 78 exhibited the highest positive GCA effects for yield in both seasons when grown under drought stress. Inbred lines CZL 02004, CZL 03002, CML 78, and CML 379 had positive GCA effects and performed consistently well under both stressed environments (low-N and drought). These inbred lines should be exploited to generate potentially good hybrids that combine tolerance to low-N with drought stress.

For the other traits, inbred line CZL 02004 performed best for the number of ears per plant (EPP) under managed drought stress, while CZL 00034 was the best for this trait under low-N. The most desirable GCA effects for ASI were observed in inbred lines CZL 02004 and CML341 under managed drought, and in CML 379, and CZL 02008 under low-N stress.

Some CML inbred lines (CML 499, CML 258, and CML 341) didn't perform as expected probably due to few crosses representing them.

The yields in the contrasting N levels differed enough to allow selection under optimum and low-N environments, with low-N causing approximately a 50% reduction in yield overall. In general, Bänziger and Laffite (1997a) concluded that selection under low-N (direct selection) is predicted to be more efficient than selection under optimum N (indirect selection) if the yield reduction in the target environment is 40% or greater. In the current experiment, it is possible that other factors (like critical N level, pH and phosphorus) might have affected the yield comparison between N-levels. A soil analysis was performed for the low-N and optimal-N experimental fields at NaCRRRI (Table 4), but it was not clear which soil factors limited the yield in the optimal-N field, or which contributed to differences between the two fields in the performance of specific hybrids.

The most promising inbred lines for contributing to breeding progress for grain yield under drought stress conditions are CML 379 and CML 78 (Tables 15, 17 & Figures 3 and 4). These lines not only have positive GCA effects for yield and EPP but also negative (ie. desirable) effects for ASI and Lsene. CZL 02004 and CZL 03002 also performed well (i.e., showed positive GCA effects for yield and EPP and negative GCA effects for ASI) and could potentially be useful parents (Musila *et al.*, 2010). These four inbred lines also could be used in other environments involved in this study (ie. non-drought stress) as they exhibited consistently desirable GCA effects and at least some of their crosses produced high grain yields when evaluated in each of those environments.

Thus, selection among inbred lines should focus on the size of GCA effects, with the choice of test environments taking into account the magnitude of NS-CGD in those

environments. The high error variances and sizeable G x E effects that were observed especially under the stressed environments limit selection effectiveness. Increasing the number of testing locations and obtaining high-quality data in each are keys to improving selection (Richards *et al.*, 2006).

Overall, yield losses (of 76%) across drought stressed conditions were primarily due to a reduction in number of ears per plant, fewer kernels per ear, and small kernel size. Similar results have been reported by Setter and Para (2010), who obtained a 62% decrease in kernel yield per plant. However, in their case, the kernel size was not significantly reduced. Lack of adequate water limits partitioning of photosynthates to the ear, with the greatest losses of grain yield occurring during ear development (Boyer and Westgate, 2004). Water deficiency also increases the anthesis-silking interval by delaying silking, resulting in lack of pollination and/or kernel abortion, causing barrenness. Thus, the most important characteristic of a cultivar to make it tolerant to drought is its ability to produce an ear under stress (Bolaños and Edmeades, 1996).

The present study revealed high correlations between grain yield with ASI and EPP under drought stressed environments (Table 19). This is in agreement with studies of Bolanos and Edmeades (1996), Richards (2006) and Musila *et al.*, (2010), who showed that high grain yield under stress depends on a short ASI. A long ASI period is a predictor of poor performance under drought.

The dependence of grain yield on EPP under drought stress results from a high proportion of ear abortion. Additionally, weak correlations observed between grain yield and Lsene under drought stress, show that this trait had little influence on grain yield.

Generally, under low-N stress, relationships between grain yield and individual secondary traits, such as ASI or EPP, were not strong enough to influence yield. Only Lyelw correlated significantly with yield. It is likely that the variations in yield observed in this environment might be related to the combined effects of a number of secondary traits. Therefore, it may not be easy to select high yielding genotypes based on individual secondary traits, a conclusion previously reported by Bänziger *et al.* (2000).

The consistency of the relationship found between grain yield and EPP and ASI across both types of stress might be explained by the strong correlation of these two traits with yield under stress conditions. These secondary traits can assist in selecting for stress tolerance as their heritability was generally high (especially ASI and Lsene) and their genetic correlation with grain yield often increases with higher levels of stress (Bolaños and Edmeades, 1996, Bänziger and Lafitte, 1997, Bänziger *et al.*, 2000 and Edmeades *et al.*, 2006). Conversely, weak correlations were found between grain yield and all secondary traits under optimum conditions (optimum N and well watered). This is in agreement with the findings of Bolanos and Edmeades (1996).

AMMI analysis based on eight total testing environments (four management environments in two seasons) revealed that there were important differences in the magnitude of G x E interaction with different environments, as shown by the direction and size of the IPCA1 scores (Fig. 5). Environments LN2 and HN1 exhibited the highest contributions to G x E interactions, and were opposite in direction, as indicated by their IPCA1 scores. There were major interactions of hybrids with seasons within the same management environments. Hybrids CML 78 x CML 379 (G43) and CZL 00003 x CML

341 (G8) yielded well across environments, but interacted negatively with some environments, as shown by their large IPCA1 scores.

The G+GE analysis resulted in a lower residual sum of squares than did AMMI, indicating that GGE better explained GxE than did AMMI in this experiment (Yan *et al.*, 2007). The 3 mega-environments (Figure 6) identified by the GGE biplot shared similar characteristics. For example, NaCRRI's well-watered optimum environment (NMWW) was in the same mega-environment as the stressed environments, presumably because it produced a low yield, despite being considered an optimum environment. Hybrid performance in different mega-environments was shown by their mean values and their IPCA scores in the specific mega-environments. Many of the hybrids did not show specific adaptation to any of the mega-environments. Hybrids 8 (CZL 00003/ CML 341), 15 (CZL 02004/ CZL 02009), and 36 (CZL 03002 x CML 78) were broadly adapted, without much interaction with any of the tested environments. Hybrids 13 (CZL 00034/ CML 379) and 43 (CML 78/ CML 379) were the best performers in the mega-environment comprising stressed environments, and thus show promise for further testing.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

The major aim of this study was to evaluate the performance of the selected group of CIMMYT maize inbred lines for use in development of superior hybrids with tolerance to drought and /or to low soil nitrogen. General (GCA) and specific combining abilities (SCA) for performance under drought and low-Nitrogen (N) stresses were determined for these inbred lines. The study also determined the effects of low-N and drought stress on the yield of single crosses of these inbred lines and evaluated whether stress-indicator traits could be used for indirect selection of yield performance under stress. Finally, the patterns of G x E interactions of hybrids were assessed under conditions of drought, low-N stress or the combined stress of drought and low-N.

The following conclusions and recommendations are made, based on the findings of this study:

- 1) Additive genetic effects for grain yield (GY), number of ears per plant (EPP) and anthesis-silking interval (ASI) were more important than non-additive genetic effects in all management environments except low-N-stress. The interaction of additive genetic effects with season and environment was generally as large as or larger than the additive effect itself, and must be considered in planning for efficient selection.
- 2) In stressed environments, hybrid means contributed only 3% to the total phenotypic variance for grain yield. Therefore, selection can be improved by including secondary traits (like ASI and leaf senescence) that correlate with yield and have high heritability.

3) Some hybrids performed well (eg. (43) CML 78/CML 379, (37) CZL 03002/CML 379 and (19) CZL 02004/CML 379) regardless of management environments. Most of those that performed well under drought also performed well under low-N stress and in the optimum environments. Some hybrids (eg. (14) CZL 02004/CZL 02008, (19) CZL 02004/CML 379, (33) CZL 01001/CML 78) combined lower-than-average yield reductions due to stress with higher-than-average yields under drought stress.

4) High correlations were observed between grain yield with EPP and ASI among genotypes across stressed environments. The best way to choose hybrids that will perform well under stress will be to select those with a high mean for yield and EPP combined with a low mean for ASI, and have parents which also have favorable GCA effects for these same traits.

5) Neither AMMI nor GGE analyses revealed a clear relationship between the test environments, season, or the presence or type of stress. However, one mega-environment identified by GGE did include all four of the stressed environments, plus the lowest yielding “optimum” environment.

6) Hybrids CZL 00034/ CML 379 and CML 78/ CML 379 gave the highest yield in this mega-environment. Hybrids CZL 00003/ CML 341, CZL 02004/ CZL 02009, and CZL 03002 x CML 78 were well adapted in all of the test environments. Hybrids CML 78/CML 379 , CZL 02004/CZL 02008, CZL 02004/CML 379, CZL 01001/CML 78 and inbred lines CZL 02004, CML 379, CML 78, and CZL 03002 could be useful for further testing in order to obtain the best hybrids for stressed environments.

7) Selection for performance in conditions of drought and low-N stress should focus on high-yielding hybrids from parents with desirable GCA values for yield, ASI, and EPP under drought stress. Three-way hybrids involving these parents may be preferable to single-cross hybrids, since they are generally more stable in stressed environments and seed can be produced at a lower cost

8) Future estimation of combining ability for performance under stress should be based on hybrids that represent as complete a set as possible from the desired mating design. In this study, some parents that did not perform as expected involved a large number of missing crosses, thus bringing into question the reliability of the GCA estimates for those inbreds.

9) Further testing and selection should be improved by efforts to increase experimental uniformity, the use of more test locations under stressed conditions, testing in farmers' fields.

10) The acidity of the optimal-N site at NaCCRI should be corrected by liming the soil prior to planting and other soil variables equalized as much as possible between the low-N site and optimal-N test sites, so that the two sites vary only in their level of N.

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APPENDIX

Appendix1 Combined analysis of variance and means for grain yield and agronomic traits across season under managed drought stress environment

Sources of variation	df	$\sqrt{\text{Mean squares}}$					F TEST
		GY† t ha-1	EPP no	ASI D	AD d	SD d	
season(S)	1	5.88*	0.014	284.03*	209.04***	1080.67**	Rep/S
Rep/S	2	0.17	0.024	3.06	1.43	5.75	P E
Hybrids(H)	42	0.59 +	0.024	24.40***	19.89***	48.15***	H x S
GCA	11	1.49*	0.061	71.98***	70.25***	158.78***	GCA x S
SCA	31	0.26	0.011	7.52	2.02	8.89	SCA x S
H x S	42	0.37*	0.019	6.15**	1.98	7.030	P E
GCA x S	11	0.41*	0.034	4.00	2.40	5.40	P E
SCA x S	31	0.36*	0.014	6.91**	1.84	7.61	P E
P E ‡	75-81	0.20	0.029	3.21	1.70	6.93	
Mean		1.4	0.6	7.3	71	79	
Min		0.2	0.3	0.5	67	71	
Max		2.6	0.8	14.4	80	88	
Cv		0.46	0.40	0.35	0.03	0.05	

+ , * , ** , *** indicates significance at 0.1, 0.05, 0.01, and 0.001 probability levels, respectively;
 †GY = grain yield; EPP = ears per plant; AD and SD = anthesis and silking date; ASI = anthesis silking interval.

‡Pooled Error.

$\sqrt{\text{Mean squares}}$ are expressed on the basis of genotype means across seasons within environments (2 reps).

Appendix2 Combined analysis of variance and means for grain yield and agronomic traits across well watered environments.

Sources of variation	df	^v Mean squares					F TEST
		GY† t ha-1	EPP no	ASI d	AD d	SD d	
Environments(E)	1	257.07**	0.228*	0.25	312.51*	312.51**	Rep/E
Rep/E	2	2.60*	0.005	5.33***	11.86***	0.55	PE
Hybrids(H)	42	2.26*	0.018	2.51***	17.62***	16.49***	H x E
GCA	11	6.58 ⁺	0.049 ⁺	6.78*	63.00***	58.68***	GCA x E
SCA	31	0.73	0.006	0.99	1.52 ⁺	1.52	SCA x E
H x E	42	1.23**	0.012 ⁺	0.59	1.59***	2.39*	PE
GCA x E	11	2.73***	0.019*	2.23***	3.42***	6.48***	PE
SCA x E	31	0.70	0.009	0.01	0.94 ⁺	0.94	PE
P E ‡	62-101	0.53	0.008	0.65	0.65	1.54	
Mean		6.2	1.1	0.9	62	61	
Min		3.9	0.9	-0.9	57	58	
Max		7.9	1.4	5.8	70	71	
Cv		0.17	0.12	1.27	0.02	0.03	

⁺, *, **, *** indicates significance at 0.1, 0.05, 0.01, and 0.001 probability levels, respectively;
†GY = grain yield; EPP = ears per plant; AD and SD = anthesis and silking date; ASI = anthesis silking interval.

‡Pooled Error.

^vMean squares are expressed on the basis of genotype means across environments (2 reps).

Appendix3 Combined analysis of variance and means for grain yield and agronomic traits across well watered and managed drought stress environments

Sources of variation	df	$\sqrt{\text{Mean squares}}$					F TEST
		GY† t ha-1	EPP no	ASI d	AD d	SD d	
WM	1	978.97	9.408*	1745.12 ⁺	4069.56 ⁺	11487.62 ⁺	WM.S
WM. Season(S)	2	131.47***	0.121*	142.14**	260.77**	699.85***	Rep/E
Rep/E	4	1.38**	0.015	4.19 ⁺	6.65***	3.15	PE
Hybrids(H)	42	1.53	0.033***	18.11*	36.32***	54.77***	H x WM
GCA	11	4.27	0.094**	55.88 ⁺	130.72***	190.84**	GCA x WM
SCA	31	0.55	0.011	4.71	2.82***	6.49	SCA x WM
H x WM	42	1.32*	0.009	8.80***	1.19	11.11**	H x WM x S
GCA x WM	11	3.81*	0.016	22.89***	2.52	26.62**	GCA x WM x S
SCA x WM	31	0.44	0.007	3.80	0.71	5.61	SCA x WM x S
H x WM x S	84	0.80***	0.016	3.82***	1.79*	5.11 ⁺	PE
GCA x WM x S	22	1.57***	0.026 ⁺	3.12 ⁺	2.91***	5.94 ⁺	PE
SCA x WM x S	62	0.53*	0.012	4.07***	1.39	4.81	PE
‡PE	143-182	0.35	0.018	1.99	1.20	3.94	
Mean		3.8	0.8	4.1	66	71	
Min		2.5	0.7	0.2	62	64	
Max		5.2	1.1	10.1	75	80	
Cv		0.22	0.24	0.49	0.02	0.04	

⁺, *, **, *** indicates significance at 0.1, 0.05, 0.01, and 0.001 probability levels, respectively;
 †GY = grain yield; EPP = ears per plant; AD and SD = anthesis and silking date; ASI = anthesis silking interval.

‡Pooled Error.

WM = environments under water management.

$\sqrt{\text{Mean squares}}$ are expressed on the basis of genotype means across seasons and environments (2reps).

Appendix4 Combined analysis of variance and means for grain yield and agronomic traits across season under low-N stress.

Sources of variation	df	√Mean squares							F TEST
		GY† t ha-1	EPP no	ASI d	AD d	SD d	Lsene Rating1-10	Lyelw Rating1-5	
Season(S)	1	49.59	0.001	17.07	148.05	266.19	11.61	0.12	Rep/S
Rep/S	2	24.54***	0.036**	10.48**	20.49 ***	54.09 ***	4.62***	1.29***	P E
Hybrid(H)	42	0.52	0.011	3.10**	14.28***	20.54 ***	0.72**	0.11***	H x S
GCA	11	0.45	0.013	7.50**	45.49***	64.91***	1.93**	0.26***	GCA x S
SCA	31	0.54	0.010	1.54	3.20	4.80	0.29	0.06	SCA x S
H x S	42	0.61**	0.013**	1.39	3.35*	3.84	0.32**	0.04+	P E
GCA x S	11	0.60*	0.011	1.05	4.99*	5.78	0.42*	0.03	P E
SCA x S	31	0.62**	0.013*	1.51	2.77	3.16	0.29*	0.04+	P E
P E‡	65-112	0.29	0.007	1.33	2.29	3.49	0.17	0.03	
Mean		2.7	1.0	2.7	68.0	70.0	2.1	1.7	
Min		1.1	0.7	1.0	63.0	65.0	1.0	1.2	
Max		3.7	1.2	6.1	73.0	78.0	3.5	2.1	
Cv		0.28	0.12	0.61	0.03	0.04	0.28	0.14	

⁺, ^{*}, ^{**}, ^{***} indicates significance at 0.1, 0.05, 0.01, and 0.001 probability levels, respectively;
†GY = grain yield; EPP = ears per plant; AD and SD = anthesis and silking date; ASI = anthesis silking interval; Lsene and Lyelw = leaf senescence and leaf yellowing.

‡Pooled Error.

√Mean squares are expressed on the basis of genotype means across seasons within environments (2 reps).

Appendix5 Combined analysis of variance and means for grain yield and agronomic traits across season under optimum N

Sources of variation	df	$\sqrt{\text{Mean squares}}$					F TEST
		GY† t ha-1	EPP no	ASI d	AD d	SD d	
Season(S)	1	3.41	0.211	4.20	86.83	129.31	Rep/S
Rep/S	2	11.95***	0.221	2.28+	76.15***	95.19***	P E
Hybrids(H)	42	0.78+	0.029**	1.71	12.03***	13.49***	H x S
GCA	11	1.12*	0.072*	2.92	36.56**	38.53***	GCA x S
SCA	31	0.66	0.014	1.28	3.32*	4.60+	SCA x S
H x S	42	0.50+	0.012	1.18	2.93***	2.58*	P E
GCA x S	11	0.39	0.018	2.00*	5.95***	2.36	P E
SCA x S	31	0.54+	0.009	0.90	1.85***	2.66+	P E
P E‡	75-112	0.34	0.193	0.92	0.64	1.68	
Mean		5.4	1.1	0.7	65.0	65.0	
Min		4.0	0.9	0.0	61.0	61.0	
Max		6.5	1.4	2.8	73.0	74.0	
Cv		0.15	0.56	1.94	0.02	0.03	

+, *, **, *** indicates significance at 0.1, 0.05, 0.01, and 0.001 probability levels, respectively;

†GY = grain yield; EPP = ears per plant; AD and SD = anthesis and silking date; ASI = anthesis silking interval.

‡Pooled Error.

√ Mean squares are expressed on the basis of genotype means across seasons within environments (2 reps).

Appendix6 Combined analysis of variance and means for grain yield and agronomic traits under optimum and low-N stress during 2009A

Sources of variation	df	^y Mean squares				
		GY [†] t ha-1	EPP no	ASI d	AD d	SD d
Environments(E)	1	232.12***	0.294***	38.89***	10.13**	87.26***
Rep/E	2	2.84***	0.128***	1.96*	52.10***	59.47***
Hybrids(H)	42	0.50*	0.012**	2.01***	18.90***	19.42***
GCA	11	0.66*	0.026***	4.15***	65.32***	66.50***
SCA	31	0.44+	0.009	0.74	2.42*	2.71*
H x E	42	0.43+	0.010*	0.92*	1.58	3.04**
GCA x E	11	0.43	0.026***	1.33*	1.36	3.45*
SCA x E	31	0.44+	0.005	0.77	1.66	2.90*
P E [‡]	74-96	0.30	0.006	0.56	1.36	1.69
Mean		3.6	1.0	1.6	66.0	67.0
Min		2.4	0.9	-0.3	61.0	62.0
Max		4.6	1.3	3.8	76.0	77.0
Cv		0.22	0.11	0.66	0.02	0.03

⁺, ^{*}, ^{**}, ^{***} indicates significance at 0.1, 0.05, 0.01, and 0.001 probability levels, respectively;

[†]GY = grain yield; EPP = ears per plant; AD and SD = anthesis and silking date; ASI = anthesis silking interval.

[‡]Pooled Error was used as F test.

^yMean squares are expressed on the basis of genotype means across environments (2 reps).

Appendix7 Combined analysis of variance and means for grain yield and agronomic traits under optimum and low-N stress during 2009B

Sources of variation	df	^v Mean squares				
		GY† t ha-1	EPP no	ASI d	AD d	SD d
Environments(E)	1	100.81***	0.951+	154.18***	608.57***	1371.09***
Rep/E	2	5.67***	0.142	10.80**	44.54***	95.81***
Hybrids(H)	42	0.81***	0.019	3.07**	9.35***	14.21***
GCA	11	0.76*	0.031	5.87***	22.42***	36.29***
SCA	31	0.83***	0.015	2.08	4.71***	6.37*
H x E	42	0.67**	0.022	1.77	2.75***	3.78
GCA x E	11	0.72*	0.031	2.11	3.89***	5.33
SCA x E	31	0.65*	0.019	1.64	2.35***	3.24
P E‡	69-106	0.33	0.243	1.55	0.24	3.40
Mean		4.5	1.1	1.8	66.0	68.0
Min		2.2	0.8	-0.2	62.0	63.0
Max		5.7	1.3	4.8	71.0	74.0
Cv		0.18	0.63	0.98	0.01	0.04

+, *, **, *** indicates significance at 0.1, 0.05, 0.01, and 0.001 probability levels, respectively;

†GY = grain yield; EPP = ears per plant; AD and SD = anthesis and silking date; ASI = anthesis silking interval.

‡Pooled Error was used as F test.

^vMean squares are expressed on the basis of genotype means across environments (2 reps).

Appendix8 Combined analysis of variance and means for grain yield and agronomic traits across optimum and low-N stress environments across seasons (2009A-2009B)

Sources of variation	df	Mean squares					F TEST
		GY† t ha-1	EPP no	ASI d	AD d	SD d	
Seasons(S)	1	39.51	0.118	2.170	4.06	12.22	Rep/seasn
Environments(E)	1	319.43***	1.151***	173.97***	387.88***	1075.08***	pooped error
S x E	1	13.50***	0.094	19.10***	230.83***	383.28***	pooped error
Rep/S	4	18.25***	0.128	6.38***	48.32***	74.64***	pooped error
Hybrids (H)	42	0.53	0.018	3.47***	23.91***	29.80***	H x S
GCA	11	0.66+	0.038*	8.45**	79.49***	97.19***	GCA x S x E
SCA	31	0.49	0.010	1.71	4.19**	5.89*	SCA x S x E
H x S	42	0.78***	0.015	1.23	4.34*	3.83*	pooped error
GCA x S	11	0.75**	0.019	1.57	8.26***	5.61*	pooped error
SCA x S	31	0.78***	0.013	1.10	2.95***	3.19	pooped error
H x E	42	0.77**	0.022**	1.34	2.40	4.23+	H x S x E
GCA x E	11	0.91*	0.047**	1.97	2.57	6.25+	GCA x S x E
SCA x E	31	0.71*	0.013	1.12	2.34	3.51	SCA x S x E
H x S x E	42	0.34	0.010	1.35	1.93*	2.60	pooped error
GCA x S x E	11	0.24	0.010	1.48	2.68*	2.53	pooped error
SCA x S x E	31	0.37	0.010	1.30	1.67	2.62	pooped error
P E‡	154-194	0.31	0.086	1.07	1.34	2.54	
Mean		4.1	1.0	1.7	66.0	68.0	
Min		3.2	0.9	0.1	62.1	63.2	
Max		4.6	1.2	3.9	73.0	75.0	
Cv		0.19	0.41	0.86	0.02	0.03	

+, *, **, *** indicates significance at 0.1, 0.05, 0.01, and 0.001 probability levels, respectively;
 †GY = grain yield; EPP = ears per plant; AD and SD = anthesis and silking date; ASI = anthesis silking interval.

‡Pooled Error.

Mean squares are expressed on the basis of genotype means across seasons and environments (2 reps).

Appendix9 Combined analysis of variance and means for grain yield and agronomic traits across low-N and drought stress environments

Sources of variation	df	$\sqrt{\text{Mean squares}}$						F TEST
		GY† t ha-1	EPP no	ASI d	AD d	SD d	Lsene Rating1-10	
SM	1	68.94	6.031**	909.028	601.42	3146.96	94.80	SM.S
SM.season(S)	2	27.74	0.007	150.55**	178.55*	673.43**	11.61	Rep/E
Rep/E	4	12.36***	0.030	6.77*	10.96***	29.92***	3.12	PE
Hybrids(H)	42	0.55	0.016	19.48***	31.00***	57.99***	0.70***	H x SM.S
GCA	11	1.18*	0.031	57.91***	106.62***	194.93***	1.92**	GCA x SM.S
SCA	31	0.33	0.010	5.84	4.17*	9.39*	0.26**	SCA x SM.S
H x SM	42	0.56	0.020	8.03**	3.17	10.70**	0.27	H x SM.S
GCA x SM	11	0.76	0.042+	21.57***	9.12*	28.75***	0.40	GCA x SM.S
SCA x SM	31	0.48	0.011	3.22	1.06	4.30	0.22	SCA x SM.S
H x SM.S	84	0.49***	0.016	3.77**	2.67*	5.44	0.32	PE
GCA x SM.S	22	0.51***	0.022	2.53	3.70*	5.60	0.42**	PE
SCA x SM.S	62	0.49*	0.014	4.21**	2.30	5.38	0.29**	PE
PE‡	140-192	0.24	0.016	2.34	1.99	5.29	0.16	
Mean		2.1	0.77	5.0	69.4	74.5	3.0	
Min		1.1	0.63	1.1	65.6	69.2	2.1	
Max		3.0	0.90	9.7	76.4	82.5	3.8	
Cv		0.33	0.23	0.43	0.03	0.04	0.19	

+, *, **, *** indicates significance at 0.1, 0.05, 0.01, and 0.001 probability levels, respectively;
 †GY = grain yield; EPP = ears per plant; AD and SD = anthesis and silking date; ASI = anthesis silking interval; Lsene, Leaf senescence.

SM = environment under stress management

‡Pooled Error.

$\sqrt{\text{Mean squares}}$ are expressed on the basis of genotype means across seasons and environments (2 reps).

Appendix10 Combined analysis of variance and means for grain yield and agronomic traits across optimum N and well watered environments.

Sources of variation	df	Mean squares					F TEST
		GY† t ha-1	EPP no	ASI d	AD d	SD d	
OM	1	26.14	0.213	2.45	383.17	334.68	OM.S
OM.season(S)	2	130.24*	0.210	2.22	199.67 ⁺	224.17 ⁺	Rep/E
Rep/E	4	7.27***	0.113	3.8**	44.01***	47.87***	PE
Hybrids(H)	42	2.06**	0.037***	2.95**	27.83***	28.43***	H x OM x S
GCA	11	5.79**	0.114***	8.01**	97.31***	94.24***	GCA x OM x S
SCA	31	0.73	0.010	1.15	3.18**	5.07**	SCA x OM x S
H x OM	42	0.99	0.009	1.27	1.81	2.79	H x OM x S
GCA x OM	11	1.91	0.007	1.69	2.24	2.97	GCA x OM x S
SCA x OM	31	0.66	0.010	1.13	1.66	2.73	SCA x OM x S
H x OM x S	84	0.87***	0.012	1.34**	2.26***	2.89***	PE
GCA x OM x S	22	1.56***	0.018	2.12***	4.69***	4.42***	PE
SCA x OM x S	62	0.62*	0.009	1.07 ⁺	1.40***	2.34***	PE
P E‡	137-182	0.43	0.096	0.82	0.64	1.60	
Mean		5.8	1.1	0.8	63	64	
Min		4.1	0.9	-0.5	59	59	
Max		7.2	1.3	3.8	72	72	
Cv		0.16	0.40	1.60	0.02	0.03	

⁺, *, **, *** indicates significance at 0.1, 0.05, 0.01, and 0.001 probability levels, respectively;
†GY = grain yield; EPP = ears per plant; AD and SD = anthesis and silking date; ASI = anthesis silking interval.

OM = environment under optimum management

‡Pooled Error.

[^]Mean squares are expressed on the basis of genotype means across seasons and environments (2 reps).

Appendix11 Hybrids and corresponding codes

No	Hybrid	Code	No	Hybrid	Code
1	CZL 00003/CZL 00034	G1	23	CZL 02008/CML 78	G23
2	CZL 00003/CZL 02004	G2	24	CZL 02008/CML 379	G24
3	CZL 00003/CZL 02008	G3	25	CZL 02009/CZL 01001	G25
4	CZL 00003/CZL 02009	G4	26	CZL 02009/CZL 03002	G26
5	CZL 00003/CZL 01001	G5	27	CZL 02009/CML78	G27
6	CZL 00003/CML 499	G6	28	CZL 02009/CML 379	G28
7	CZL 0003/CML 258	G7	29	CZL 01001/CZL O3OO2	G29
8	CZL 00003/CML 341	G8	30	CZL 01001/CML 499	G30
9	CZL 00003/CML 78	G9	31	CZL 01001/CML 258	G31
10	CZL 00034/CZL 02004	G10	32	CZL 01001/CML 341	G32
11	CZL 00034/CZL 03002	G11	33	CZL 01001/CML 78	G33
12	CZL 00034/CML 78	G12	34	CZL 01001/CML 379	G34
13	CZL 00034/CML 379	G13	35	CZL 03002/CML 341	G35
14	CZL 02004/CZL02008	G14	36	CZL 03002/CM 78	G36
15	CZL 02004/CZL 02009	G15	37	CZL 03002/CML 379	G37
16	CZL 02004/CZL 01001	G16	38	CML 499/CML 258	G38
17	CZL 02004/CZL 03002	G17	39	CML 499/CML 379	G39
18	CZL 02004/CML 78	G18	40	CML 258/CML 78	G40
19	CZL 02004/CML 379	G19	41	CML 341/CML 78	G41
20	CZL 02008/CZL 01001	G20	42	CML 341/CML 379	G42
21	CZL 02008/CZL 03002	G21	43	CML 78/CML 379	G43
22	CZL 02008/CML341	G22	44	Check	G44