

Heterosis, Combining ability and Tester Identification for Maize (*Zea mays*) Adapted to Low Nitrogen Conditions.

By Cathrine Ziyomo



**A thesis submitted in partial fulfillment of the requirements for the degree of
Master of Science In Crop Science (Plant Breeding)**

Department of crop Science

Faculty of Agriculture
University of Zimbabwe

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A Thesis

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Submitted to the Department of Crop Science, Faculty of Agriculture of the University of Zimbabwe in
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MASTER OF SCIENCE (MSc.) IN CROP SCIENCE (Plant Breeding)

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ABSTRACT

The production of high yielding single cross maize testers with good combining ability the potential to discriminate materials under evaluation across diverse environments, is important in breeding programs. Testers have a lifespan and the continuous introduction of new and diverse germplasm into a breeding program renders the old testers irrelevant and obsolete in many cases. The objective of the study was to identify new single cross testers to replace CML312/CML442 (Heterotic group A) and CML395/CML444 (Heterotic group B), currently being used in the maize breeding program at CIMMYT Zimbabwe. Ten inbred lines comprising of 4 lines used in the current testers and 6 new elite lines were crossed in a diallel mating design to produce all possible F₁ combinations excluding reciprocals. The resultant single cross hybrids were evaluated under optimum moisture and fertilization conditions and low nitrogen stress in Zimbabwe during the 2006/2007 main growing season. General combining abilities (GCA) and specific combining abilities (SCA) were calculated using Griffing's Method 4 model 1. The single cross CML312/CZL04006 was identified as the best candidate that could replace CML312/CML442 for heterotic group A. CZL04006 had a positive and significant General Combining Ability (GCA) of 0.49(±0.64) while CML312 had a GCA value of 0.21(±0.26). The Specific Combining Ability (SCA) for this cross was -1.61 (±0.12) lines. There was relatively high intra group high parent heterosis for this cross (348%). The single cross CML444/CZL068 performed better than the current B tester, CML395/CML444 for grain yield across the two environments. CZL068 had the second highest GCA estimate (1.24, SE ±0.31) after CML444 (± 0.54). The SCA for the cross was -0.06 (±0.24). The intra group heterosis realized was 316%. CML444 had the highest GCA value of 1.71(±0.54). The two promising single cross testers identified in this study performed well under optimum and low nitrogen stress conditions, had good combining ability for yield across the tested environments, and had high heterosis. Further testing is needed particularly under drought stress to establish combining abilities and performance as well as to determine stability under a wide range of environments.

DECLARATION

The thesis study was carried out at the International Maize and Wheat Improvement Centre (CIMMYT-Zimbabwe) in collaboration with the University of Zimbabwe under the supervision of Dr Cosmos. Magorokosho, Dr Charles Mutengwa and, Mrs. Shorai. Dari.

Research presented in this thesis represents original work by the author and has never been submitted in any form for degree or diploma to any University. Where use has been made of the work of others, it is duly acknowledged in the text.

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LIST OF ABBREVIATIONS

CIMMYT	International Maize and Wheat Improvement Centre
AREX	Agricultural Research and Extension
FAOSTAT	Food and Agricultural Organization Statistical Database
ASI	Anthesis Silking Interval
AD	Anthesis Days
GY	Grain Yield
PH	Plant Height
N	Nitrogen
EPO	Ear Position
EPP	Ears Per Plant
EH	Ear Height
SEN	Senescence
CHL	Chlorophyll
RL	Root Lodging
SL	Stem Lodging
KW	Kernel Weight
masl	Meters Above Sea Level
T/ha	Tonnes Per Hectare
GCA	General Combining Ability
SCA	Specific Combining Ability
DNA	Deoxyribo Nucleic Acid
HG	Heterotic Group

CHAPTER 1

INTRODUCTION

1.1 Introduction

Maize is an important food crop for southern Africa as reflected by the large area devoted to its production and the high human consumption levels. Maize production in Africa in 2004 was estimated to be 41,6 million metric tones of which 27,4 million metric tones were produced in sub-Saharan Africa (FAOSTAT, 2005). Maize provides 50% of the calories in diets of southern Africa, 30% in eastern Africa and 15% in west and central Africa (FAOSTAT, 2005). In southern Africa, per capita annual consumption of maize averages more than 100kg in several countries (Lesotho, 149kg; Malawi, 181kg; South Africa, 195kg; Swaziland, 138kg; Zambia, 168kg; and Zimbabwe, 153kg (CIMMYT, 1999).

Despite the crop's significance as a major food crop, grain yields across Africa remain low (average < 1, 2 t/ha) mainly due to drought and low soil fertility (CIMMYT, 2001). These two abiotic stresses threaten maize production, food security and economic growth in eastern and southern Africa (Banziger and Diallo, 2004). In Africa, the crop is grown by small and medium scale farmers who cultivate 10ha or less, under extremely low input systems (DeVries and Toenniessen, 2001). Losses due to drought have been estimated to be around 17% annually with regional losses reaching 70% under extreme conditions compared to well-watered conditions (Edmeades *et al.*, 2001).

Low nitrogen in soils is an important yield-limiting factor frequently found in farmers' fields in the tropics where fertilizer is not commonly used and is rapidly mineralized if used (Banziger and Lafitte, 1997). The incidence of abiotic stress on maize may increase due to global climatic changes, displacement of maize to marginal environments by high value crops, declines in soil organic matter, reduced soil fertility and water holding capacity (Banziger and Cooper, 2001).

The development of maize cultivars with high and stable grain yields under drought and low nitrogen is therefore an important priority for breeders as this may be one of the few affordable opportunities for the resource poor farmers to increase and sustain maize yield levels under the stress conditions that frequently occur in their fields.

The International Maize and Wheat Improvement Center (CIMMYT), an international non-profit making organization, is involved in strengthening and augmenting plant breeding efforts of various private sector and national breeding programs in pursuit of seed and ultimately food security in many tropical areas of the world. Some of CIMMYT's efforts are directed towards improving maize varieties for drought tolerance and low soil fertility stress tolerance. In 1997 CIMMYT initiated a product oriented breeding program for southern Africa, targeted at improving maize for drought prone environments particularly at lower yield levels (Banziger *et al.*, 2002). Since then, significant progress has been made in improving maize for stress tolerance with the formation of hybrids, inbred lines and open pollinated varieties.

Information is still limited on combining ability of maize inbred lines and on choice of the best testers to use when developing stress tolerant three way and double cross hybrids. Understanding the genetic basis for hybrid performance under abiotic stresses and identifying parental inbred lines that form superior hybrids is crucial in designing appropriate breeding strategies. Further advancement in the yield of maize requires certain information regarding the nature of combining ability of the parents available for use in the breeding program as well as the nature of gene action involved in expression of both quantitative and qualitative traits of economic importance.

General combining ability (GCA) and specific combining ability (SCA) effects are important indicators of the potential value of inbred lines in hybrid combinations and in grouping materials into heterotic groups. The use of heterotic groups, when aided with good testers in a breeding program can result in the production of high yielding hybrids. Testers of hybrid value or heterosis between parental inbred lines can increase the efficiency of hybrid breeding programs. A diallel mating design is a useful tool for

testing and analyzing a number of lines in all possible combinations. Results of a diallel can be used to identify inbred lines with superior combining ability that may be used as testers in a breeding program, and for identifying superior crosses that may be candidates for single cross hybrids.

CIMMYT Zimbabwe's maize breeding program in southern Africa uses single cross testers since the aim of the hybrid-breeding program is to produce three way and double cross hybrids. Single crosses are vigorous in growth, produce more pollen than inbred lines and give high seed yields. Rawlings and Thompson (1962) suggested that an ideal tester should maximize the differences among the genotypes being tested. Single crosses CML312/CML442 (designated as Heterotic Group A) and CML395/CML444 (designated as Heterotic Group B) are the current testers being used for early generation screening of maize lines at CIMMYT Zimbabwe.

The production of high yielding single cross maize testers, which have good combining ability, have high intra- heterotic group heterosis and can discriminate materials under evaluation across diverse environments, is important in a breeding program. However, as a breeding program progresses, the germplasm become more higher yielding rendering the testers relatively low yielding. In addition, the continuous introduction of new and diverse genotypes into germplasm pools as well as challenges like new diseases or the change of environments may render the old testers irrelevant necessitating the need for identifying new ones.

The study aimed at identifying single cross testers that are stress tolerant, have high and stable yields across environments and are good combiners, which can replace the testers currently, used by the CIMMYT Zimbabwe maize program. The study also aimed at generating additional information on the combining ability and heterosis of the inbred lines under study as well as evaluating the agronomic performance of the single cross hybrids under different environments. The identification of new testers may result in better-discriminating testers under stress conditions and improved selection of good three way hybrids in the testing phase of the breeding program.

1.2 Objectives of the study

1. To determine the heterotic relationships between CIMMYT's four inbred lines that are currently being used in single cross testers and six new inbred lines that are being proposed for potential parents of single cross testers.
2. To estimate heterosis, general and specific combining abilities of CIMMYT's maize inbred lines for grain yield and other agronomic traits especially under abiotic stress conditions.
3. To determine the relative performance of new maize single cross hybrids relative to the performance of single crosses currently being used at CIMMYT Zimbabwe.

Hypotheses tested

1. Recently bred inbred lines can form single cross testers that can better discriminate germplasm under evaluation as compared to the old testers.
2. Recently bred inbred lines that are potential parents for new single cross testers show high heterosis and combining abilities as compared to inbred lines used as parents of single cross testers at CIMMYT Zimbabwe.
3. New single cross hybrids formed in this study can outperform the single cross hybrids currently used as testers at CIMMYT Zimbabwe.

CHAPTER 2

LITERATURE REVIEW

2.1 Maize production and consumption trends in Zimbabwe

Maize is the staple food of Zimbabwe and the per capita annual consumption averages 153kg (CIMMYT, 2001). It ranks first in terms of production level as well as the consumption level. In Zimbabwe it accounts for 70% of the total area under cereals with 60% of the annual production coming from the small-scale communal farmers (CIMMYT, 2001; Pingali, 2001). The country requires 1.8 metric tones of maize and 300 000t as national strategic reserve per annum. This annual requirement is divided into the following proportions, 64% for human consumption, 22% for livestock and poultry and 14% for other industrial uses (Mashingaidze, 2006). White maize is preferred for human consumption while yellow maize is preferred for livestock feeds.

Owing to the seasonal rainfall distribution and its inadequacy over much of the country, rainfall is the overriding factor determining maize production and also determining suitability of land for agricultural use in Zimbabwe (Mashingaidze, 2006). The minimum amount of rainfall adequate for maize production is considered to be 700mm. However, the country is situated in a zone of erratic rainfall with only 35% of the area receiving 700mm or more per annum (Ministry of Lands and Rural Resettlement, 2000). The short rainy season frequently not exceeding four months imposes limitations on the permissible delay in planting if yield level reduction is to be avoided. The reliability of rainfall differs in many parts of the country with greater reliability in the Eastern part of the country. Production in the smallholder sector is severely constrained by technical, financial, marketing and managerial factors and the unreliability of rainfall since smallholder farmers cannot afford irrigation (Ministry of Lands and Rural Resettlement, 2000). Supplementary irrigation is mostly found in the large-scale commercial areas and not in the smallholder-farming sector. There is no

doubt irrigation is of crucial importance to agriculture and the national economy at large.

The high capital cost of irrigation infrastructure means that the production of maize by smallholder farmers will continue to depend on the limited rainfall and the success of cropping will depend highly on management techniques as well as breeding. The development of tropical maize cultivars with high and stable grain yields under drought and low nitrogen conditions has potential to narrow the yield gap between research stations and the farmer's field. The average grain yield is 1,3t/ha for the country, whereas the average for research stations is above 7t/ha (CIMMYT, 1999).

2.2 Combining ability, heterosis and heterotic group studies

Combining ability tests are necessary since it is not possible to predict the performance of hybrids from visually assessing or measuring their *per se* performance or of the component inbred lines or genotypes. Information on combining ability of germplasm under evaluation can also help in the exploitation of heterosis.

2.2.1 Combining ability

Combining ability of inbred lines is determinant of the potential usefulness of an inbred line in hybrid combinations and the final evaluation of inbred lines can be best determined by hybrid performance. According to Allard (1960) combining ability is a measure of the value of a genotype based on the performance of their offspring produced in some definite mating system. The genotypes used could be populations, varieties or inbred lines. The diallel mating design originally proposed by Griffing (1956a) is useful for estimating GCA and SCA effects for a set of genotypes and their implications in plant breeding.

Sprague and Tutum (1942) refined the concept of combining ability to produce the two expressions of GCA and SCA. GCA is the average performance of a line in hybrid combinations expressed as a deviation from the overall mean of all crosses made from

other parental lines (Falconer, 1981). These deviations can either be positive or negative. A positive deviation can be favorable or unfavorable depending on the trait under consideration. Negative values are not desirable for yield traits but for days to flowering where earliness is required they are favorable. Genetically, GCA is primarily associated with alleles which are additive in their effects whereas SCA is attributed to the non-additive genetic portion of the total genetic effects (Rojas and Sprague, 1952). Additive effects are the predictable portion of the genetic effects and are therefore useful to plant breeders. GCA tests are used for preliminary screening of lines from a larger number of lines in a breeding program. They are also used to identify the type of gene action governing traits of interest. A high GCA estimate is indicative of additive gene action. Genotypes with poor GCA are discarded. GCA effects quantitatively measure the comparative performance of parents and cross combinations in relation to one another.

Any particular cross has an expected value, which is the sum of the GCA of the two parental lines. The cross may deviate from the expected value to a greater or lesser extent and this deviation is called the SCA of the two lines in combination (Falconer, 1989). Sprague and Tatum (1942) defined SCA as those instances in which certain hybrid combinations are either better or poorer than would be expected on the average performance of the parent inbred lines included in the crosses. SCA is used to indicate the value of superior genotype combinations especially in intra group crosses. The SCA measurement represents the final stage in the selection of inbred lines as it identifies specific inbred lines to use in hybrid formation. A high SCA measure indicates non-additive gene action. In addition SCA estimates can be used to determine heterotic relationships among different genotypes. Lines from different heterotic groups, which give high positive SCA estimates, are said to be complimentary to each other (Hallauer and Miranda, 1981).

2.2.2 Heterosis

Heterosis has been defined as superiority of the F_1 hybrid over both its parents (Singh, 2003). Generally heterosis is manifested as an increase in vigor, size, growth rate, yield or some other characteristic and this superiority is estimated over the average of the two

parents (the mid-parent value). The manifestation of heterosis depends on genetic divergence of the two parental populations (Hallauer and Miranda, 1988). Genetic divergence of the parental varieties is inferred from the heterotic patterns manifested in a series of crosses. The benefits of crossing genetically divergent parents include heterosis and genotype complementarity, which is the optimum combination of genotypes to use their strengths and hide their weaknesses.

The effective use of heterosis involves the development of populations with high combining ability (Griffings, 1956a; Vasal *et al.*, 1992). In maize, inbred lines are low yielding while hybrids exhibit a high degree of heterosis for yield as well as other agronomic traits like plant height and days to maturity (Duvick, 1999). However, high yielding hybrids owe their high yield levels not only to heterosis but also to other heritable factors that are not necessarily influenced by heterosis. Heterosis is not only dependent on the parent combinations but also on the effect of environmental conditions as well as the trait under consideration (Chapman *et al.*, 2000).

Two major types of heterosis have been defined (Lamkey and Edwards, 1999) according to the types of parents involved in making the hybrids and these are mid-parent or average heterosis and high parent or better parent heterosis. Mid-parent heterosis is the heterosis observed when two random populations are crossed together and is used for selecting populations for recurrent selection programs and to determine the amount of relationship among cultivars. This is the one commonly used and the formula for the conditions necessary for heterosis of quantitatively inherited traits is:

$$H = \sum dy^2,$$

where H is the mid-parent (average heterosis), d is the effects due to dominance and y^2 (which determines the amount of heterosis expressed in the cross) is the square of the difference in allele frequency between the lines or the populations (Falconer, 1981). The differences in the allele frequency and the dominance of loci of inbred lines and cultivars are generally not known. Therefore experimental data, obtained from hybrids and their respective parents are the only sources available to determine the levels of heterosis expressed in hybrids (Falconer, 1981).

High parent heterosis is the difference between the mean of the F₁ hybrid and mean of the highest performing parent making up the hybrid (Lamkey and Edwards, 1999). It is then from the measurements of heterosis that the heterotic relationships are determined. In some cases however, the hybrid may be inferior to the weaker parent. Often the superiority of the F₁ is estimated over the superior parent and it may also happen that a hybrid inherits and exhibits the worst qualities of each of its parents, or is inferior to both. The hybrid should perform over both its parents for the heterosis to be of any value to the breeder and the degree of heterosis depends on the relative performance of inbred parents and the corresponding hybrids.

Falconer and Mackay (1996) defined heterosis as the converse of inbreeding depression, which is the difference between hybrid value for one trait, and the mean value of the two parents for the same trait. Inbreeding depression is described as the depressing effect in the expression of traits which comes about due to an increase in the level of homozygosity as the plants are inbred and is manifested as a reduction in plant vigour, size of various plant parts, reduced yield levels and reduced reproductive capacity.

Heterosis is usually small or absent in traits that are influenced by additive genetic effects (Hallauer and Miranda, 1988). In the most basic form, additive gene action is the summation of many genes “adding up” together to bring about a total result. Heterosis is then one of several genetic effects that are part of the non-additive genetic effects. The magnitude of additive gene effects is an important determinant of whether a trait is heritable. For tropical maize, Betran *et al.* (2003) reported extremely high expression of heterosis under stress, especially under severe drought stress because of the poor performance of the inbred lines under these conditions. It is generally believed that inbred lines with superior yields under drought and low N will result in superior hybrids under these conditions, even though correlations between inbred parent performance and hybrid performance are relatively weak (Vasal *et al.*, 1997).

2.2.3 Heterotic groups

A heterotic group is a group of related or unrelated genotypes from the same or different populations which display similar combining ability when crossed with genotypes from other germplasm groups (Warburton *et al.*, 2002). A heterotic pattern is a specific pair of heterotic groups (or lines), which express high heterosis in hybrid combinations (Warburton *et al.*, 2002). Heterotic groups are important as they allow better exploitation of germplasm in a hybrid-breeding program.

Some of the methods that can be employed in developing heterotic groups include making crosses in a diallel fashion (Hayman, 1954), making crosses using the North Carolina Design II (Robinson and Cockerham, 1958) mating design and using DNA markers to classify the germplasm (Melchinger, 1999). The choice of which method to use is governed by the source germplasm under study as well as the resources available.

A heterotic group contains different genotypes, which show similar heterosis because of similar allelic frequencies. It represents broad sources of germplasm, which exhibit optimum heterosis when crosses are made between the groups. The parents of a single cross cultivar typically belong to opposite heterotic groups (Duvick, 1999). A heterotic group comprises a set of inbred lines that have similar performance when crossed with inbred lines from another heterotic group and usually these will show little or no heterosis when crossed to each other because they are generally closely related.

The inbred lines within a heterotic group are often related due to advanced cycle breeding. The heterotic groups that compliment each other comprise a heterotic pattern that is, specific crosses between genotypes, which show high levels of heterosis. Classifying inbred lines into heterotic groups is critical to determine the potential usefulness of the lines for the development of high yielding hybrids and synthetic varieties. Although heterotic patterns are very critical for maximizing the potential expression of heterosis in hybrids, they have not been well established and improved in a systematic manner by the majority of maize improvement programs in the tropics. A

higher level of diversity in tropical maize has made it relatively difficult to find uniform heterotic groups (Warburton *et al.*, 2002).

Heterotic groups are desirable because of the need to include related lines in the same single cross parents of the double crosses and they are equally useful in pedigree breeding programs for developing inbred lines as parents of single cross hybrids. In recent years heterotic groups have become more distinct and refined because of emphasis of selection within elite line crosses for developing parents of single crosses and with the assistance of molecular markers to resolve questionable assignments. Heterotic groups are then used to classify germplasm according to the expression of heterosis (Melchinger, 1999).

The distinction between heterotic groups is not absolute, because a heterotic group can be made from germplasm of distinct heterotic groups. Instead, heterotic groups are said to be open ended since more materials of tested affinities can be added to them. These new materials introduce important traits like resistance to disease and drought tolerance that are needed as challenges (drought, disease) in the production of maize emerge (Hallauer, 1999). An example is the temperate maize single cross B73 and Mo73. The B73 line was derived from the Reid yellow dent (now Stiff Stalk) heterotic group while the line Mo73 was derived from the cross 187-2 crossed to C103. Line 187-2 was derived from the improved line Krug of the same Reid yellow dent group as B73 (Hallauer *et al.*, 1988).

CIMMYT developed a number of heterotic groups from some of the broad groups to suit its lowland tropical, subtropical and highland maize breeding programs. In eastern and southern Africa, the heterotic groups are based on Southern Cross (SC), Salisbury White (N3), and K64r/M162W and Natal Potchefstroom Pear Elite Selection (NPPES) varieties. The varieties SC, N3 and NPP ES were developed from varieties imported from the USA while K64r is a direct import from the USA. M162W is an improved version of K64r (Mickelson *et al.*, 2001). In the CIMMYT programs of Southern Africa, the two heterotic groups are A and B. Group A includes the following germplasm: Tuxpeno, Reid Yellow Dent and N3. Group B has ETO, Lancaster Sure Crop and SC germplasm (Mickelson,

2003). Inbred lines representing these groups are CML442 and CML312 (Group A) and CML395 and CML444 (Group B).

2.3 Testers in breeding programs

Testers are genotypes of good GCA and well defined heterotic groups, which are used for identifying (selecting) superior genotypes to use in breeding programs. They are very important in determining the heterotic alliance of new inbred lines as well as evaluating the breeding values of genotypes for population improvement. Inbred lines, single cross hybrids or heterogeneous materials can be used as testers. Rawlings and Thompson (1962) pointed out that a good tester should be able to provide precision in “discriminating” among genotypes (separating the good from the bad effectively) and should provide information that classifies the merit of lines and maximizes genetic gain. In general a good tester should be poor in the traits for which the lines are to be analyzed but should have broad adaptation to the target environment.

The procedure for identifying superior genotypes by using testers entails evaluation of testcrosses for GCA effects. The choice of what type of tester (broad based or narrow based) to use in a breeding program depends on the availability of testers, type of materials under test and type of hybrids for which the lines are to be used. In selecting for GCA, a broad based heterogeneous population is used as a tester and when selecting for SCA a narrow genetic base (inbred or single cross) is used. Testers will change with the objective of a program and the types of hybrids developed but all the same, the testers should be highly adapted to environmental variability. Studies by several researchers show that inbred line testers give relatively more information for GCA than SCA (Hallauer and Miranda, 1988).

The choice of initial tester to use is based on experience with most commercial hybrid development programs using inbred parents with proven hybrid performance. Breeders use information on the pedigree of the genotypes being tested along with the knowledge of the performance of the tester with the parents of these genotypes in making this choice. No single tester fulfills all these requirements in all given circumstances since the value

of a tester is determined to a considerable extent by the use to be made of a particular group of lines.

The best compromise for an inbred tester is to select a successful line unrelated to the inbred lines being tested and adapted to the target environment for the hybrid. At the onset of any hybrid evaluation the breeder needs to determine the relative combining ability for the new inbred lines. In terms of practicality there is need to use the same testers for evaluating combining ability under drought or low N stressed conditions, as well as under optimum (well watered and well fertilized conditions).

2.4 Breeding and screening for abiotic stress

Drought and low soil fertility are the stresses threatening maize production, food security and economic growth in sub Saharan Africa (Banziger and Diallo, 2004). Strategies to improve the tolerance of maize to these two abiotic stresses have been put in place mainly targeting the dynamics of the maize crop during the flowering period and the incorporation of secondary traits can also increase breeding progress.

2.4.1 Effects of drought and low nitrogen on yield

The most important abiotic stresses limiting maize production in eastern and southern Africa are drought and low soil fertility and in the tropics, these two stresses occur in association (Banziger *et al.*, 1997). Maize production in sub Saharan Africa shows variability through time (Hassan *et al.*, 2001) and this is attributed to abiotic stress (Bolanos and Edmeades, 1993a). The stress may increase due to global climatic changes and the displacement of maize to marginal environments by high value crops and partly due to reduction in soil organic matter, leading to reduction in soil fertility and water holding capacity (Banziger *et al.*, 1997). This fertility and water availability varies greatly in many farmers' fields especially in the tropics and that requires a single variety to be able to withstand a wide range of drought stress and nitrogen availability (Banziger *et al.*, 2000).

Most tropical maize is produced under rain-fed conditions and many of the maize varieties grown in eastern and southern Africa are susceptible to drought. The high cost of irrigation means that the production of maize by smallholder farmers will continue to depend on the limited rainfall and the success in cropping will depend on breeding as well as better crop management techniques.

2.4.2 Secondary traits in selection

Secondary traits are certain plant characteristics of adaptive value which are usually less relevant under non-stressed conditions but become very important for yield under drought and low N conditions. These traits are termed secondary traits in contrast to the trait of primary interest – yield. Under stressed conditions, the genetic variance for yield decrease more rapidly than the environmental variance among plots making it difficult to detect differences between genotypes (Lafitte *et al.*, 1997). However, under these conditions the genetic variance for secondary traits increases (remains high) or is reduced less than that of yield and the genetic correlation between grain yield and these traits increases sharply (Banziger and Lafitte, 1997) making these traits of great importance in improving the selection efficiency. Ideally a secondary trait should be genetically associated with yield under the type of stress, highly heritable, genetically variable, cheap and easy to measure and should not be associated with a yield penalty under unstressed conditions (Edmeades *et al.*, 1995).

Physiologists and ideotype breeders have advocated the incorporation of secondary traits into breeding programs for the following reasons: 1) they can improve the precision with which drought or low N tolerant genotypes are identified, compared to measuring only grain yield under these stresses, 2) they can demonstrate the degree to which a crop was stressed by drought or low N, 3) if observed before or at flowering, they can be used for selecting desirable crossing parents, 4) if observed before maturity, they can be used for preliminary selection when turn around time between seasons is short. If included in selection with grain yield, these traits can increase gains compared with selection for grain yield alone (Banziger and Lafitte, 1997).

Evaluation of the adaptive value of a trait begins by showing that it is related to yield under stress in a field environment. Under drought, the following traits have been advocated for as aides in selection; a reduced Anthesis- Silking interval (ASI), low leaf senescence, high leaf chlorophyll content, leaf rolling, tassel size and ears per plant. For low N, the traits are ears per plant, leaf senescence and ASI. Bolanos and Edmeades (1996) pointed out that the correlation analysis between yield and secondary traits must be interpreted with care, because results are often confounded by genetic differences among genotypes for other traits or by the presence of outliers.

2.4.3 Drought stress at flowering

Drought stress is unpredictable, occurring with variable intensity at any crop development stage from germination to physiological maturity and varying year by year. Under well-watered conditions, silks emerge at about the same time pollen is shed but when drought stress occurs just before or during flowering in maize, it causes delay in silk emergence which subsequently increases the anthesis-silking interval (ASI) or asynchrony in floral development, loss of pollen viability, failure of fertilization as well as embryo abortion. Studies on the timing of drought stress have indicated that flowering is the most sensitive stage for yield determination in maize, and losses in grain yield and kernels per plant can exceed 50% when drought coincides with this period (Boyer and McPherson, 1976; Grant *et al.*, 1989, Simmonds, 1991).

Maize, a cross pollinated crop, is more sensitive than other cereals to water deficits and high temperatures at flowering because anthers and silks are separated in space by about 1m and stigmas are fully exposed to the environment (Edmeades and Bolanos, 1993). In addition, a large proportion of maize kernels develop synchronously, usually on a single ear, and stress at a critical stage may cause complete ear abortion. A commonly observed phenomenon under drought is the lengthening of the ASI which is usually caused by a delay in silk emergence relative to the emergence of anthers, the latter being little affected by drought (Edmeades and Bolanos, 1993; Fischer *et al.*, 1983). Delayed silk emergence maybe due to a reduced rate of silk elongation, a process that is strongly affected by plant water status (Westgate and Boyer, 1997).

2.4.4 Breeding strategies for drought prone environments

There are three main strategies that can be utilized in breeding for drought prone environments and these are drought escape, drought tolerance and selecting for high yield potential. Drought escape describes the situation where an otherwise drought susceptible variety performs well under drought simply by avoiding the period of drought (Singh, 2003). Breeding may aim at developing cultivars that escape drought by being early in maturity so as to complete their life cycle within a given season length. Selection for earliness matches the phenology of the crop to the pattern of water availability.

Drought tolerance is a mechanism that enables crops to suffer a minimum loss and therefore yield a good harvest. This is important in areas where periodic droughts occur during the flowering phase of the maize crops. Drought tolerant cultivars are characterized by increased production under drought. Mere survival with no grain yield is of little importance except at the seedling stage since the ability of a genotype to produce a grain-bearing ear is the most important indicator of drought tolerance (Banziger *et al.*, 2000).

Drought tolerance can be approached through crop yield stability under stress, response to stress indicative of tolerance, the biology underlining the responses and the genes and alleles governing the presence or expression of these responses. Variation among genotypes exists in their tolerance to drought and responses to drought have shown that to be heritable (Blum, 1988; Clark, 1982). Grain yield and secondary traits are employed in conjunction with each other in screening for drought tolerance.

High yield potential (including heterosis) is a constitutive trait that often gives increased yield under moderate levels of drought that is when drought stress reduces yields by less than 50% (Banziger *et al.*, 2000). Selection for high yield potential entails genetically correlating yields of the same cultivar grown in two different environments of one stressed and the other well watered. If the genetic correlation between yields is positive and statistically significant, selection for yield potential will produce a variety tolerant to

moderate levels of stress (Banziger *et al.*, 2000). If on the other hand the correlation is not significant or is zero (0) or is negative (-), selection for yield potential alone will not affect drought tolerance (Banziger *et al.*, 2000).

2.4.5 Drought screening

Rosielle and Hamblin (1981) suggested that breeding under high yielding; non-stressed conditions might not be the best approach to increasing yields where severe abiotic stresses are encountered in the target environment since the best performing genotypes under optimal conditions are not necessarily the best under stress. Breeders should therefore include selection under the target environment. Screening procedures for genetic differences in drought tolerance include 1) defining practical objectives of the screening process 2) selection of environments and stress occurrence to be targeted in the program 3) the design and operation of field physical facilities and experimental methods to apply uniform, repeatable drought stress.

Several workers (Atlin and Frey, 1990; Ceccarell *et al.*, 1992; Banziger *et al.*, 1997) have concluded from experiments that breeding progress can be increased if abiotic stress in the target environment is included during selection. Edmeades *et al.*, 1993 carried out trials to assess the potential of recurrent selections under managed drought stress in improving grain yield in tropical maize which is targeted to be grown under stressed conditions.

The materials used for the experiment were one early maturing and three late maturing male populations adapted to tropical lowlands. Secondary traits were used to help with selection. Selections were for progenies with high yield, reduced ASI, increased relative stem leaf extension rate, cooler canopy temperature, increased leaf angle and increased stay green. There were eight cycles of recurrent full sib or up to four cycles of recurrent S1 selection practiced on this material.

The gain in yield under full sib selection was between 73kg/ha/year and 144kg/ha/year and a mean of 111kg/ha/year. The trials were subjected to levels of stress, which gave

yields between 1,5t/ha and 2,4t/ha. Gains under non-stressed conditions (well watered whose potential yield was between 5,6t/ha and 8t/ha) ranged from 38kg/ha/year and 108kg/ha/year with a mean of 59kg/ha/year. There was a clear better gain when selections were done under stressed conditions compared to non-stressed condition. It was then concluded that it is possible to increase grain yields through selection under severe drought stress.

2.5 Breeding strategies for low nitrogen environments

The development of low N tolerant varieties is of high priority for breeders targeting the tropical areas especially sub-Saharan Africa. Low N tolerant germplasm is characterized by staying green for a longer period of time (Lafitte and Banziger, 1997; Lafitte and Edmeades, 1994) and uses N in the form of chlorophyll and photosynthetic enzymes for carbohydrate production for a longer period of time. For effective identification of low N tolerant genotypes breeding strategies make use of selections under conditions of severe low N stress.

2.5.1 Development of low N tolerant varieties

Low N stress on maize causes yield reduction of up to 50% due to a reduction in photosynthesis. Photosynthesis is reduced as a result of reduction in leaf area development, accelerated leaf senescence and a reduced rate of photosynthesis itself. It is estimated that 50% (Banziger *et al.*, 2000) of all leaf N is involved in photosynthesis. Leaf senescence due to a short supply of N begins with the bottom leaves because the plant relocates N from the bottom older tissue to the younger leaves and grains up the plant. An increase in the root/shoot ratio and delayed pollen shedding and silk emergence when stress is severe is also observed. The delay in silking is relatively more than the delay in pollen shedding resulting in a lengthened ASI especially when the N stress coincides with flowering. This delay in silking is correlated with barrenness.

If stress in the target environment (the environment in which the selected genotype will be grown) gives yields in which are less than 40%(ideally 25%-35%) of the yield

obtained under well fertilized conditions (Banziger *et al.*, 2000) then the stress tolerant genotypes can be identified. The reason being that the correlation between genotype performance under low N and well fertilized conditions diminishes with an increase in severity of stress. This therefore means that there is no relationship between genotype performances under well fertilized environments and environments severely stressed for N (Banziger *et al.*, 1997).

2.5.2 Low N screening

Breeding strategies for low N environments aim at selecting genotypes under conditions of severe N stress coupled with the use of secondary traits. The secondary traits recommended in order of diminishing importance are as follows: grain yield, ears per plant, leaf senescence and ASI. High grain weight is desirable when selecting low N tolerant genotypes. Measurement of the grain weight is done on shelled grain adjusted for moisture. The grain weight is then used to calculate grain yield. Selection for ears per plant is aimed at identifying genotypes with no barren plants or genotypes with at least one ear. An ear is defined as a cob having at least one fully developed grain. Leaf senescence is visually scored on 2-3 occasions, 7-10 days apart during the latter part of the filling period. For ASI, selection for this trait is aimed at a reduced or negative value.

Banziger *et al.*, 1997 conducted trials to assess the efficiency of high N selection environments for improving maize targeted for low N environments at CIMMYT-Mexico. Fourteen replicated trials were grown under low N (no nitrogen applied) and high (200kg/ha nitrogen) nitrogen. Results from these trials showed that: a) the genetic correlation between grain yields under low N and high N was generally positive, b) selection under high N for performance under low N was predicted significantly less efficient than selection under low N when relative yield reduction due to N stress exceeded 40%, c) genetic correlation decreased with increasing relative yield reduction under low N. These results indicated that as the specific adaptation to either low or high N became more important, the low N and high N experiment differed in grain yield. The conclusion from the results was that low N selection environments should be included in order to maximize selection gains for environments when N stress is important.

2.6 Diallel mating design

The diallel cross was defined as all possible crosses among a group of parents (Griffings, 1956a) including the parents themselves. With n parents, there would be n^2 families (Jinks and Hayman, 1953; Jinks, 1956) and the n^2 families are a “complete Diallel cross”(Crumpacker and Allard, 1962; Baker, 1978). If the reciprocal crosses and parents are excluded, giving $n(n - 1)/2$ families, the result is a “half diallel”(Morley-Jones 1965). A “modified diallel” is one in which the parents are not included (Griffing, 1956b). A partial diallel includes fewer than the $n(n-1)/2$ crosses, but the crosses are so arranged that valid statistical analysis and interpretation are possible (Kempthorne and Curnow, 1961; Gardner and Eberhart, 1966; Gilbert, 1958).

Diallel analyses were developed based on quantitative inheritance of traits in populations, using covariance's between relatives (Fischer, 1988; Hayman, 1954) and the utilization of this analysis for identification of superior combinations has been a common practice in maize breeding programs. Wright (1985) pointed out that there are three possible levels of diallel analysis and these are: 1) Estimation of general and specific combining ability, 2) Estimation of genetic variance components and 3) A complete genetic analysis.

Griffing (1956c) proposed a diallel technique for determining the combining ability of lines and characterizing the nature and extent of gene action in both plants and animals and he proposed four methods of diallel crossing. Method 1 includes the parents, reciprocal crosses and the F_1 . Method 2 involves the parents and the F_1 s but no reciprocal crosses. Method 3 includes the F_1 s and reciprocal crosses but no parents. Method 4, which is commonly called a half diallel, only includes the F_1 s with neither parents nor reciprocal crosses.

Criticisms of diallel analysis, and perception of abuse generally arise from the interpretations made from the results. Appropriateness of the various kinds of diallel crossing methods depends on the experimental material, the trait under evaluation and the

objectives of the experiment (Griffing, 1956a). For example, when the trait is influenced by maternal effects or cytoplasmic inheritance, Method 1 or Method 3 with modifications (Borges, 1987) may be used.

Griffing analysis allows the option to test for fixed effects (Model 1) and random effects (Model 2). The choice of a fixed or random effects model will determine if individual GCA and SCA effects for parents and crosses should be estimated or if the variance of these effects should be estimated. The fixed model analysis yields considerable information about a fixed set of parents and no inference can be made from that set about the whole population. The information can be useful for the selection of parents that have good GCA in a series of crosses and good SCA for specific pairs of parents (Hallauer and Miranda, 1988). The random effects model is usually used when parents are sampled from a population and the information obtained will be relevant to the whole population.

CHAPTER 3

MATERIALS AND METHODS

3.1 Germplasm

Ten elite white maize inbred lines (Table 3.1) originating from the CIMMYT maize breeding program were crossed in the winter of 2006 at Muzarabani in a diallel mating design. The inbred lines were developed using the pedigree method and were phenotypically uniform. The inbred lines used in the study were drawn from the two heterotic groups, designated A and B which are used at CIMMYT Zimbabwe and were of different maturity groups (early, intermediate and late). A total of 41 single cross hybrids were obtained after bulking the reciprocal crosses; four crosses were missed completely.

3.1.1 Experimental design

An alpha lattice design for incomplete blocks with two replicates was used for each experiment (Patterson and Williams, 1976) during the summer of 2006/7 season. The plots were planted in single row plots spaced 0.75m apart and the in-row spacing was 0.25m. At CIMMYT Harare, two seeds were sown by hand per planting station and seedlings were thinned to one plant per station at four weeks after planting, resulting in a final density of 53,000 plants per ha. At ART farm, four seeds were sown by hand at each planting station and seedlings were thinned to two plants per station spaced 0.5m apart to give a final plant density of 53, 000 plants per ha. The plots were 4 metres long and the total area was 3.875m².

Parent	Pedigree	Source	Maturity	HG	Principal characteristics
CML312	CML312	POP500	Intermediate	A	Semi flint, susceptible to MSV
CML395	CML395	IITA	Late	B	GLS resistant, flint
CML442	CML442	Recycled	Intermediate	A	Dent, tolerant to drought and low N stress
CML444	CML444	POP.43CP	Late	B	Semi dent, tolerant to drought and low N stress
CML502	CML502	RCWQ	Intermediate	A/B	Semi flint, QPM, GLS resistant, MSV susceptible
CZL1	[CML442/CML197//[TUXPSEQ]C1F2/P49-SR]F2-45-7-3-2-BBB]-2-1-1-1-1-B*4-B	Recycled	Early	B	
CZL2	[(CML395/CML444)-B-4-1-3-1-B/CML395//DTPWC8F31-1-1-2-2]-5-1-2-2-BB-B	Recycled	Late	B	Semi flint
CML312SR	MAS[MSR/312]-117-2-2-1-B*4-B-B	MSR	Intermediate	A	Semi flint, MSV resistant
CZL068	[LZ956441/LZ966205]-B-3-4-4-B-5-BBBBB-B	Recycled	Late	B	Flint
CZL04006	ZM621A-10-1-1-1-2-BBBBBB-B-B	Population ZM621	Intermediate	A	Flint, tolerant to drought stress

Key: HG Heterotic Group

Table 3.1 Germplasm used in the experiment

3.1.3 Test locations and environments

The inbred parental lines were planted at Muzarabani in the winter of 2006 and then the single crosses were planted at ART farm and CIMMYT Zimbabwe research station. In making the crosses, each line was used both as a male and as a female according to a diallel mating design scheme (Hallauer and Miranda, 1988). Some crosses failed to produce seed due to poor germination and stand establishment of some of the parental inbred lines and so the diallel dataset was incomplete. After bulking the crosses, 4 crosses were missed completely resulting in 41 unique single cross hybrids. The crosses that were missed were CML312/CML312SR, CML312/CZL2, CML395/CZL068, and CML442/CZL04006.

Site	Management	Altitude (masl)	Latitude	Longitude	Rainfall (per yr)
Muzarabani	Optimal	420	20.02 ⁰ S	29.84 ⁰ E	320mm
Harare (CIMMYT)	Low nitrogen	1455	17.80 ⁰ S	18.32 ⁰ E	800mm
ART farm	Optimal	1468	17.80 ⁰ S	18.32 ⁰ E	820mm

Masl = metres above sea level

Table 3.2 Evaluation sites for the single cross hybrids and inbred lines

3.2 Low Nitrogen stress management

The Harare low N site at CIMMYT Zimbabwe has been depleted of mineral nitrogen by continuously growing maize for several years without adding any N fertilizer and removing the maize stover every season. As a result N supply to the crop becomes dependant on soil mineralisation. The low N block soil analysis results showed 4ppm N in the top 30cm which translates to approximately 30kg N per hectare. This is about 25% of the required N under optimal conditions at this location. Single super phosphate fertilizer was applied at a rate of 400kg/ha (14%P₂O₅; 7% K₂O) to supply the crop with the other macronutrients (phosphorous and potassium).

3.1 Crop husbandry and data collected

At the optimum conditions site (well watered and well fertilized) the crop was irrigated and fertilized to avoid stress. Herbicides and pesticides were used to keep the trials weed free and pest free. At CIMMYT Harare, the same was done except that no N-containing fertilizer was applied.

3.2.1 Field management

Ploughing was done using a tractor drawn heavy disc plough for both sites in the summer of 2006. A pre-marked chain was used to mark planting stations at spacings of 0.75m between rows and 0.25m within rows at CIMMYT Zimbabwe and 0.75m between rows and 0.5m within rows at ART farm. Trials of inbred parents were planted adjacent to the hybrid trial at both sites to enable calculation of heterosis.

3.2.2 Fertilizer application and water management

A basal application of 400kg/ha of compound Z fertilizer (8%N: 14%P₂O₅: 7% K₂O:0.8% Zn) was broadcast and disc incorporated by a tractor at ART Farm. Top dressing using ammonium nitrate with (34.5% N) was applied at these locations at four weeks and then eight weeks after planting at a rate of 200kg/ha. Supplementary irrigation using an overhead sprinkler irrigation system was used as and when required to avoid stressing the crop at both locations.

3.2.3 Weed management

The nursery for the diallel as well as the trials were kept weed free throughout the season through herbicide. A mixture of atrazine (Atrazine WP), dual (Metalochlor) and gramoxone (Paraquat) was used to control weeds at a rate of 4.5, 1.8, and 1.0 L/ha respectively as a pre-emergent control. Herbicides were applied using a 500L spray tank with a 10m wide boom with 20 nozzles mounted on a pick up truck at CIMMYT Harare. Three weeks after emergence, basagran (Bentazone) was applied to control nut-sedge

(*Cyperus rotundas*) and other broad leaf weeds. At four weeks after emergence, Bentazone was applied again to control all weeds. From seven weeks onwards, weeds were controlled by hand hoeing.

3.2.4 Pest management

Cutworm (*Agrotis ipsilon*) was controlled using Karate (Lambda cyhalothrin), which was band applied (30cm from crop row) at a rate of 100mL/ha in 200L of water. Maize stalk borer (*Buseola fusca*) was controlled using Endosulfan granules (Thionex) which were hand-applied every 10 days alternating with dipterex at 2kg/ha. Maize streak virus disease was controlled by applying Carbofuran (Curator) at a ratio of 3 parts of chemical to 4 parts sand in the planting hole to kill the *Cicadulina* leafhoppers, which are the vectors of the disease.

3.2.5 Data collected

Field data for number of days to flowering (at 50% anthesis and 50% silking), plant and ear height, standability, leaf senescence, turcicum disease scores, chlorophyll content, a thousand kernel weight, field and grain weight were recorded. Some derived traits such as anthesis-silking interval (ASI), lodging percentage, ears per plant (EPP) and yield per hectare (at 12.5% moisture adjustment) were also calculated using Fieldbook software(Vivek *et al.*, 2007). Table 3.3 shows the traits that were measured and how they were measured.

Abbreviation	Trait	Units	Procedure of measurement
SD	Silking date	days	Number of days from planting when 50% of the plants in a plot have extruded silks
ASI	Anthesis-silking interval	days	Derived from anthesis date and silking date as follows: ASI= SD - AD
EPP	Ears per plant	0-1	Calculated as a ratio of the number of ears with at least one fully developed grain divided by the number of harvested plants.
PH	Plant height	cm	Measured as the height between the base of a plant and the insertion of the first tassel branch.
EH	Ear height	cm	Measured as the height between the base of a plant to the insertion of the top ear.
EPO	Ear position	0-1	Calculated as EH divided by PH.
RL	Root lodging	%	Measured as a percentage of plants that showed lodging by being inclined 45°.
SL	Stem lodging	%	Measured as a percentage of plants that were broken below the ear.
SEN	Leaf senescence	1-10	Number of leaves that are yellow below the ear as a percentage
ET	Disease Score		Taken using a 1-5 score with 1 being resistant and 5 being susceptible
MOI	Grain moisture	%	Percentage moisture as determined by the moisture meter
GY	Grain yield	t/ha	Calculated from shelled grain weight per plot adjusted to 12.5% grain moisture.
CHL	Chlorophyll content	Spad units	Chlorophyll content as determined by a chlorophyll spad meter
1000KW	A thousand kernel weight	grams	Weight of a thousand kernels
TEX	Grain texture	1-5	Rated on a scale 1-5 with one being flint and 5 dent
HC	Husk cover	%	Percentage of plants with ears that are not completely covered by the husks

Table 3.3 Field measurements

3.3 Statistical analysis

Analysis of variance for each environment and adjusted means were computed with the REML tool (Vivek *et al.*, 2007). Genotypes were considered as fixed effects and replications and blocks as random effects. Combined analysis across environments was also computed using Proc GLM in SAS (SAS, 2001) and this enabled the testcrosses to be assessed under stress and non-stress conditions.

3.3.1 Combining ability analyses

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Since some of the crosses were not successful, during the analysis, the crosses CML312/CML312SR, CML312/CZL068, CML442/CZL04006 and CML395/CZL2 were estimated as missing crosses using the following formula:

$$[(n-1)(Ta + Tb)] - 2T / n^2 - 5n + 6$$

where: n is the number of inbred lines in the set, Ta and Tb are totals of performances from different traits including the missing cross, $2T$ is the grand total from all the crosses for each trait in the set (Eckhardt, 1942).

Data were analyzed according to Griffing's method IV (excluding reciprocals and parents) (Griffing, 1956b) for a fixed effects model (Model I) using DIALLEL-SAS 05 (Zhang *et al.*, 2005). Agronomic evaluation trials of the inbred parents were planted adjacent to the hybrid trials to enable calculation of heterosis. The performance of the testcrosses was based on the GCA estimates of the individual inbred lines and SCA estimates of the crosses. The combining ability analysis was according to the mathematical model:

$$X_{ij} = \mu + g_i + g_j + s_{ij} + 1/bc (\sum \sum e_{ijkl}), \quad 1j = 1 \dots p, \quad k = 1 \dots b, \quad l = 1 \dots c,$$

Where μ is the population mean, g_i and g_j are the GCA effects, and s_{ij} the SCA effect

Such that $s_{ij} = s_{ji}$ and e_{ijkl} is the error effect peculiar to the $ijkl$ th observation. The restrictions imposed on the combining ability effects are: $\sum g_i = 0$, and $\sum s_{ij} = 0$ for each j (Griffing, 1956a).

Source	df	MS	E (MS) Model 1
Replications	$r-1$		
Crosses	$\{n(n-1)/2\}-1$	M_2	$\delta^2 + r\delta^2_c$
GCA	$n-1$	M_{21}	$\delta^2 + (n-2)(1/n-1)\Sigma g^2$
SCA $I < J$	$n(n-3)/2$	M_{22}	$\delta^2 + [2/[n(n-3)] \Sigma \Sigma s_{ij}^2$
Error	$(r-1)\{n [(n-1)/2]-1\}$	M_1	δ^2
Total	$r[n(n-1)/2]-1$		

r and n refer to the number of replications and parents respectively

Table 3.4 Diallel analysis of variance for a fixed model (Model 1)

CHAPTER 4

RESULTS

4.1 Analyses of variance and combining ability

Analysis of variance was conducted for all the traits under study for both locations and a combined analysis of variance across the two sites was also done.

4.1.1 Optimal environment (Art Farm)

At this location, highly significant differences ($P < 0.001$) among single cross hybrids and among inbred lines were observed for all traits except ASI (Table 4.1). Highly significant differences were observed for AD, GY, EH, 1000KW and EPP, and moderately significant ($P < 0.01$) were observed for AD and MOI (Table 4.1). ASI, CHL and PH did not show any significant differences between the entries. The average grain yield for the hybrid trial was 8.36 t/ha whilst it was 3.67t/ha for the inbred line trial (Appendix A1). The highest yielding single cross hybrid was CML444 x CZL068 (Table 4.1) and crosses in which CML444 was involved, had high yields. The late maturing hybrids (with days to anthesis > 75 and grain moisture $> 13\%$) had hybrids that were high yielding while a similar trend was also observed for the earlier maturing hybrids.

Combining ability analysis revealed highly significant mean squares for GY, AD, ASI, EH, EPO, MOI, TEX, EPP and CHL but not for PH and 1000KW. Both GCA and SCA contributed significantly to the entries' variation with the exception of GCA for PH and EPP and also the exception of SCA for ASI, PH, EPO, MOI and TEX. The magnitude of GCA mean squares for all traits was more than twofold for all the traits in comparison with SCA mean squares. The contributions from GCA to SCA mean squares of the entries showed that additive effects (GCA) were more important than non-additive effects (SCA) for all traits although there were differences in the magnitude.

Mean squares												
Source	df	GY	AD	ASI	PH	EH	EPO	MOI	TEX	1000KW	EPP	CHL
		t/ha	days	days	cm	cm	cm	%	1-10	g	0-1	spads
Entry	44	8.39***	16.35*	19	840*	554***	0.01**	6.07*	1.95**	6793***	0.105***	46.76
GCA	9	15.2***	49.34*	45**	2490	2239***	0.08***	11.9**	4.85***	17764	0.306**	92.22**
SCA	35	6.63*	7.86***	12	415	121	0.02	4.56	1.04	3972***	0.05***	35.07
Error	44	1.5	2.46	13	46	91	0.01	3.05	0.95	855	0.011	3.763
Mean		8.36	72	0.5	251	133	0.52	15.95	2	326	1.04	73.66
Max		13.35	79	5	291	1167	0.57	20.41	4.5	446	1.69	65.35
Min		7.86	66	-3.5	228	83	0.44	13.25	1	114	0.47	35.67
LSD (0.05)		0.62	0.75	1.73	3.23	4.5	0.017	0.83	0.466	13.98	0.05	0.92

Table 4.1 Combined analysis of variance and mean squares for grain yield and agronomic traits for optimum conditions.

*, **, *** Indicates significance at 0.05, 0.01, and 0.001 probability levels respectively.

GY = grain yield; AD = anthesis days; ASI = anthesis- silking interval; PH = plant height; EH = ear height; EPO = ear position; MOI = grain moisture; CHL = leaf chlorophyll content; TEX = texture; EPP = ears per plant; TEX = grain texture; 1000kw = a thousand kernel weight.

4.1.2 Low N stress environment (CIMMYT Zimbabwe)

At this location, highly significant differences ($p < 0.001$) among single cross hybrids were observed for AD, ASI, PH, EH, MOI, 1000KW and CHL (Table 4.2). The mean yield for the hybrid trial was 2.54t/ha (Table 4.2) and 0.87t/ha for the inbred line trial (Appendix AI). The highest yielding single cross hybrid was the single cross CML395 x CZL068 (5.12t/ha) and CML444 was the in-bred line which had the highest yield (1.63t/ha). Grain yields for hybrids under low N were on average 65% of the hybrid yields obtained under optimal conditions and for the inbred lines it was almost 25% of the average under optimal conditions (Appendix AI). Compared with the hybrids, the inbred lines were relatively more sensitive to low N stress with some not even being able to have a grain bearing ear hence the low average yields that were not significantly different from each other (Appendix A1).

The low nitrogen stress caused a slight increase in days to anthesis (73 days) for the hybrids compared to the optimal environment (72 days). A notable difference due to stress was detected for the anthesis silking interval between the two environments. There were highly significant differences among entries ($p < 0.001$) for the secondary traits ASI, CHL and SEN whilst the differences among entries for EPO were not significant (Table 4.2). Mean squares for the grain yield components (1000kw and EPP) were significant although differences for 1000kw were highly significant ($p < 0.001$).

The analysis of variance indicated that both GCA and SCA effects were significant sources of variation GY, ASI, EH, EPO, EPP, SEN and CHL and that only GCA contributed significantly to the variation for MOI and 1000kw whilst SCA was significant for AD and PH (Table 4.2). GCA mean squares were consistently higher than SCA mean squares for all the traits and partitioning the entries mean squares showed that GCA explained a major proportion of the entries variation for most traits. The magnitude of the differences between GCA mean squares and SCA mean squares for the differences was lower for the low N environment as compared to the optimal environment for all the traits except chlorophyll content.

Mean squares												
Source	df	GY	AD	ASI	PH	EH	EPO	MOI	SEN	1000KW	EPP	CHL
		t/ha	days	cm	cm	cm	cm	%	1-10	g	0.1	spads
Entry	44	1.7**	14.15***	19.8***	734***	303.7***	0.002	5.98***	064***	2655***	0.04**	73.26***
GCA	9	3.33***	38.77	28.84***	2008	807.3***	0.05**	1.12***	1.24**	10446***	0.05**	178***
SCA	35	1.2*	6.48**	17.49**	402.69***	173.22*	0.001	0.29	0.47*	651	0.04*	46*
Error	89	0.82	114.822	4.73	133.11	71.5	0.001	0.23	0.28	480.5	0.02	19.53
Mean		2.54	73	3	170.4	85	0.46	11.6	5.15	204.8	0.87	31.63
Max		4.51	81	20	227.5	125	0.55	12.65	6.125	295.8	1.18	45.4
Min		1.21	71	-0.5	97.5	50	0.41	10.2	3.875	152.9	0.48	21.03
LSD(0.05)		0.433	0.77	1.03	5.51	4.04	0.01	0.23	0.255	10.47	0.07	2.11

Table 4.2 Combined analysis of variance and mean squares for grain yield and agronomic traits for low N conditions.

*, **, *** Indicates significance at 0.05, 0.01, and 0.001 probability levels respectively.

GY = grain yield; AD = anthesis days; ASI = anthesis silking interval; PH = plant height; EH = ear height; EPO = ear position; MOI = grain moisture; CHL = leaf chlorophyll content; TEX = texture; EPP = ears per plant; SEN = leaf senescence; 1000kw = a thousand kernel weight.

4.1.3 Combined analysis across environments

The five single cross hybrids that had the best yields for the across site analysis were CML312/CML444 (8.82 t/ha), CML395/CML312SR (8.35), CML395/CZL04006 (7.89 t/ha), CML444/CZL068 (7.61) and CZL1/CZL068 (7.66 t/ha). Of these hybrids CML395/CML312SR and CML444/CZL068 showed stability across the two environments as they did not show a high yield penalty when they were grown under stress. Entry and entry by environment interactions were highly significant ($p < 0.001$) for grain yields of the single cross hybrids (Table 4.3) and the mean squares for entry, entry x environment and pooled error were 318, 2.84 and 6.21 respectively. Mean grain yields for the hybrids ranged from 1.65 t/ha (CML312SR/CZL2) to 8.82 t/ha (CML444/CZL068).

The results shown in Table 4.3 indicate that there were highly significant differences between the entry and environment interactions for GY, ASI, PH and moderately significant for EPO, 1000kw, EPP and CHL and no significant differences for AD EH and MOI. Partitioning of the entry variation into GCA and SCA showed that GCA explained most of the variation for entries as indicated by the high mean squares for the traits. There were significant fluctuations in the magnitudes of the GCA and SCA across the two environments as shown by the significance of the E x GCA and E x SCA interactions (Table 4.3) for most traits. Further partitioning of the entry by environment interaction into GCA x E and SCA x E showed that both GCA x E and SCA x E differences were highly significant for GY and EH whilst only GCA x E differences were significant for AD PH MOI and variation due to SCA by E was highly significant for ASI EH 1000KW and CHL.

For most traits, there were no significant interactions between SCA and the environment compared with GCA and GCA mean squares were consistently higher than SCA mean squares for all the traits analyzed as was observed under optimal and low N conditions. GCA interactions with the environment had strong significance for PH, EH, CHL, and 1000kw and a moderately significant interaction for GY, AD, and MOI.

Mean squares											
Source	df	GY	AD	ASI	PH	EH	EPO	MOI	1000KW	EPP	CHL
		t/ha	days	days	cm	cm	cm	%	g	0-1	spads
Source											
Environ(E)	1	2784***	1547***	36.25	457***	3925*	45.17	3878*	79.38***	2.87***	4956***
Entry	44	318***	42.91	621.4***	263.32***	18.91	6.65	3.13*	15.76**	20.51*	30.08***
GCA	9	14.11***	438.75**	2391*	6291***	339.51**	1.47*	2.52**	2.39***	33.74**	203*
SCA	35	6.23***	12.94***	191.63*	83.51	2.07	0.95	5.47**	9.25**	40.21	1.85***
Entry x (E)	44	2.84***	11.38	161.4***	10.25***	154.26	54.36*	1.19	272.65*	1.56*	171*
GCA x E	9	4.43**	19.24**	3.42	25.46***	41.21***	4.98	22.68**	336.19	6.39*	5.67***
SCA x E	35	1.67*	4.68	1.63**	10.41	191.47***	82.45	4.9	87.76**	10.25	7.39
Error	88	1.16	2.51	121.35	51.93	2.36	74.12	3.52	6.32	4.87	4.86
Mean		6.21	72.4	1.4	248.4	125.8	0.51	12.8	356.4	1.06	57.4
Max		8.82	80.4	7.2	146.7	130.25	0.62	10.1	452.6	1.82	66.7
Min		1.65	65.4	-1.5	289.3	65.4	0.43	23.5	175.6	0.47	34.7
LSD		0.79	3.8	2.62	18.5	9.91	10.21	14.21	3.26	0.22	3.58

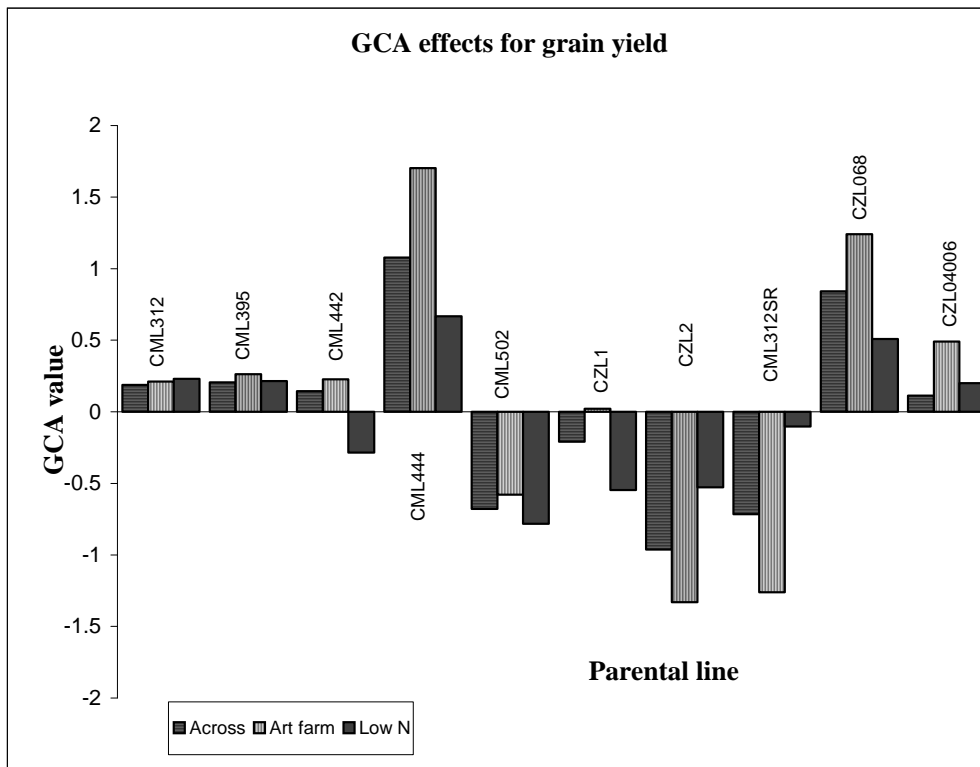
Table 4.3 Combined analysis of variance and mean squares for grain yield and agronomic traits across two environments.

*, **, *** Indicates significance at 0.005, 0.01, and 0.001 probability levels respectively.

GY = grain yield; AD = anthesis days; ASI = anthesis silking interval; PH = plant height; EH = ear height; EPO = ear position; MOI = grain moisture; CHL = leaf chlorophyll content; TEX, texture; EPP, ears per plant; 1000kw = a thousand kernel weight.

4.2 General Combining Ability effects for grain yield

Five of the parental lines used in this study had positive GCA effects estimates for grain yield for both environments (optimal and low N) as well as for the across environment analysis (Figure 4.1). Of these five, three of them (CML 312, CML395, CML444) are being used in single cross testers for the CIMMYT Zimbabwe maize program. The fourth one, CZL068 which is in designated heterotic group B, had the second highest GCA effects estimate for yield under optimal conditions (1.24). The fifth one, CZL04006 derived from ZM621 Population A and in heterotic group A, had GCA estimates that were significantly higher than those of the other inbred lines in heterotic group A for both environments. Of the lines currently being used in single cross testers, only CML442, had a negative GCA estimate (Figure 4.1) under low N conditions.



Standard error ± 0.56

Figure 4.1 GCA effects for grain yield

CML312SR, the maize streak resistant version of CML312, had consistently negative GCA estimates across both sites and had the poorest GCA estimate (-1.26) for the optimal environment. In contrast, this line had high-inbred line *per se* performance as indicated by the high yield estimates shown across the two environments (Appendix AII). The other three lines (CML502, CZL1, CZL2) consistently had negative GCA estimates for both experimental sites as well as the across site. Most of the inbred lines had higher GCA estimates in terms of magnitude for the optimal conditions as compared to the low N environment (Figure 4.1).

4.3 GCA effects for other agronomic traits and secondary traits

Parental inbred lines that had positive GCA effects for AD were late maturing (Appendix A) and also had positive GCA estimates for grain yield (Figure 4.1). CML444, which had the highest GCA estimate for yield, also had the highest GCA estimate for AD. However, this was not the case with CZL068, which despite [having](#) the second highest GCA estimate for yield (Figure 4.1), had a negative estimate (-2.79) for AD (Table 4.4). Despite the difference, these two lines were the best for GCA and hybrids in which they were involved were high yielding. CML395, CML442, CZL1 and CZL068 had positive GCA effects for AD and they are intermediate for maturity. Most of the lines that had a smaller value for ASI had negative GCA values for ASI. CML395, CML442, CML502 and CZL2 had positive GCA estimates vales for ASI (Table 4.4).

There was no consistent association between GY and EPP in terms of magnitude of GCA values as some lines that had high, significant and positive GCA estimates for GY had negative values for EPP (Table 4.4). However, there was some degree of consistency for 1000KW. The highest positive value for EPP was seen in CZL1 whilst the least was observed in CZL2 (-0.18). CML442 had the highest positive GCA estimate (1.26) for grain texture, as this line is heavily dent whilst CZL2 had the highest negative value for grain texture (Table 4.4). Parents that had high and positive GCA estimates for CHL also

had high and positive GCA estimates for grain yield except for CML444 and CML395, which had negative GCA, estimates for CHL.

Parent	GY	AD	ASI	PH	EH	EPO	HC	MOI	CHL	1000KW	ET	TEX	EPP	SEN
CML312	0.21	-1.04	-0.75	5.98	-1.73	-0.01	2.94	0.29	4.98	19.62	-0.08	0.14	0.13	-0.17
CML395	0.26	0.53	2.31	9.48	9.96	0.02	-0.61	0.35	-2.36	45.48	0.01	-0.55	-0.12	-0.11
CML442	0.23	0.28	0.31	-0.78	1.84	0.01	-0.41	0.23	1.28	-16.5	0.28	1.26	-0.07	0.44
CML444	1.71	3.03	-1.62	17.2	23.58	0.05	-0.7	0.11	-0.87	8.38	-0.08	-0.02	0.12	-0.13
CML502	-1.08	1.34	0.13	7.98	3.59	-0.06	0.36	-1.27	-1.77	-61.7	0.19	-0.51	-0.16	0.39
CZL1	0.02	1.46	-0.69	3.67	-3.6	-0.02	-1.95	1.63	-0.09	8.29	-0.15	-0.19	-0.18	-0.37
CZL2	-1.34	-1.10	3.56	-16.40	-8.2	0.005	0.19	0.77	-2.90	-31.2	-0.27	-0.61	-0.18	0.28
CML312SR	-1.26	-2.03	-0.93	-23.9	-22.3	-0.03	0.56	-0.62	1.14	-15.5	-0.08	-0.24	-0.15	0.07
CZL068	1.24	-2.79	-0.68	-6.65	-1.91	-0.02	1.28	-0.54	2.04	43.65	-0.11	0.51	0.12	-0.24
CZL04006	0.49	0.34	-1.63	3.41	-1.16	-0.01	-1.59	-0.96	-1.44	2.38	0.32	0.22	0.05	-0.17
MEAN	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SE (g_i-g_j)	0.56	0.75	1.73	3.23	4.56	0.012	1.52	0.835	0.93	13.98	0.08	0.47	0.05	0.63

Table 4.4 GCA effects of parental lines for different agronomic traits

Key – PH: Plant height (cm); GY: Grain yield (t/ha); ASI: Anthesis-silking interval (days); EH: Ear height (cm); AD: Anthesis date (days); EPP: Ears per plant (count); EPO: Ear position (0-1); 1000KW: thousand kernel weight; CHL: Leaf chlorophyll content; ET: Ear turcicum (Scale 1-10); TEX: Grain texture (Scale 1-5); HC: Husk cover (%).

Six of the inbred lines had negative GCA estimate values for SEN (Table 4.4), indicating some level of stress tolerance and the values ranged from -0.37 (CZL1) to 0.39 (CML502) (Table 4.4). CML442 had the highest positive GCA value (0.438) for SEN while the least value was observed in CZL2. Lines with the poorest *per se* performance values for GCA estimates for SEN were those with positive values. The lines showed very little differences in their GCA estimates EPO and ET.

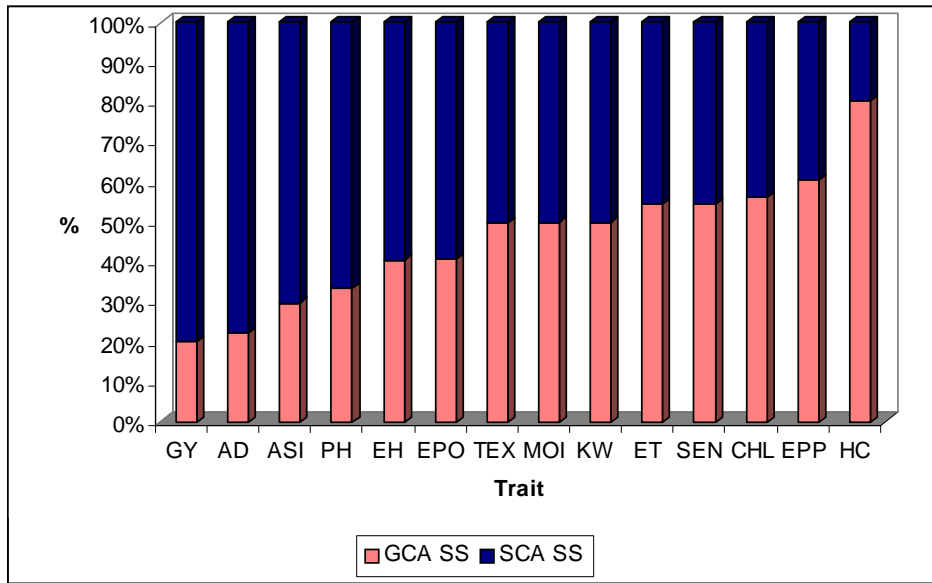


Figure 4.2 Contribution % of GCA and SCA to entry sums of squares for the different traits

Partitioning of the hybrid sums of squares for crosses showed that GCA accounted for greater than 70% of the variation among the hybrids for husk cover and similarly SCA accounted for greater than 70% of the variation among the hybrids for GY and AD (Figure 4.2). There was a predominance of GCA sums of squares for SEN, CHL, EPP and HC. Whilst SCA was sizable for ASI, PH, EH and EPO its contribution to the hybrids' variation for TEX, and KW was almost similar to that of GCA.

The 0.21 ratio of GCA sums of squares (Figure 4.2) substantiates the relatively more important role of SCA in determining yield levels for hybrids

4.4 SCA effects for grain yield

The magnitude and direction of SCA effects varied considerably among the crosses with 21 crosses having a positive SCA estimate and 24 having a negative SCA estimate (Table 4.5). Cross combinations from diverse genetic backgrounds (Heterotic group A versus group B) had positive SCA effects with high performance while the reverse held true for closely related parents. SCA effects were significant for distantly related (heterotic group A x heterotic group B) and non significant for within heterotic group crosses. While SCA was important for this study, its contribution was less than that of GCA for most traits. The single cross CML312/CML312SR had a negative SCA estimate (-0.12) since they are derived from the same source germplasm with the difference being that CML312SR has been improved for maize streak resistance. The SCA values ranged from -6.85 (CML312SR x CZL2) to 2.69 (CML395 x CML312SR).

Parent	CML395	CML442	CML444	CML502	CZL1	CZL2	CML312SR	CZL068	CZL04006
CML312	-2.06	-0.37	2.09	-0.82	1.01	1.31	-0.12	-0.26	-1.61
CML395		0.11	-1.21	-0.89	-0.25	-0.01	2.69	-0.33	1.94
CML442			-0.41	-1.01	-1.89	1.42	0.62	-1.42	2.24
CML444				0.67	-0.27	1.65	0.39	-0.16	-2.87
CML502					2.01	0.81	-0.77	-0.24	0.26
CZL1						0.08	0.64	-0.33	-0.99
CZL2							-6.85	1.44	0.15
CML312SR								1.84	1.51
CZL068									-0.63

SE ($s_{ij} - s_{ik}$) = ± 1.54

Table 4.5 SCA effects of grain yield in t/ha of 45 hybrids from the 10-parent diallel

The single cross testers currently being used at CIMMYT Zimbabwe had negative SCA estimates with CML442/CML312 for heterotic group A having an SCA estimate of -0.37 and CML395/CML444 for heterotic group B having an SCA estimate of -1.21 (Table 4.5). The four inbred lines in the single cross testers consistently showed heterotic behavior (for crosses between and within heterotic group) that agreed with their pedigree

information. Both single cross testers (CML312/CML442 and CML395/CML444) were among the best yielding hybrids for grain yield for the two environments. However for most crosses, SCA effects were important in predicting F1 hybrid performance and the effects were directly related to high yields. Most crosses with consistent positive SCA effects for yield ranked first to fifteenth in yield and also had higher yields in comparison with the grand mean yield of 8.36t/ha (optimal conditions Table 4.1) and 2.54 t/ha (Low N conditions (Table 4.2). Similarly those with consistent negative SCA effects ranked sixteenth to forty- fifth and had lower yields than those with positive SCA values except for CML442/CML444, CML312/CZL068, CML395/CZL068 and CML312/CML395.

4.5 Heterosis estimates for grain yield

Heterosis was estimated as High Parent Heterosis (HPH) for both the optimal environment and the low N environment and Table 4.6 shows some of the heterosis estimates. Although SCA effects were negative for within group crosses, the heterosis estimates were high for most crosses.

Higher heterosis was generally observed under the low N environment compared to the optimal environment and the estimates ranged from -76.15 (CZL2 x CML312SR) to 372.52 (CML312 x CML502) for the optimal environment and from -66.6 (CZL2 x CML312SR) to 613.4 (CML502 x CZL1)(Appendix EI). Table 4.6 shows that it was not always the case that inbred lines that had high and positive GCAs and SCAs in their crosses ultimately had high heterosis percentages. The single cross between the parents that had the highest GCAs (CML444 x CZL068) had high heterosis (316%), which was not the highest for the hybrids under observation and also had a negative SCA estimate.

Hybrid	Optimal (GY, t/ha)			Low N (GY, t/ha)			SCA effects		GCA effects
	GY t/ha	HPYld t/ha	% Het t/ha	GY t/ha	HPYld t/ha	% Het	P1	P2	
CML395xCM312SR	8.31	4.29	260.89	4.46	0.87	418.7	2.69	0.21	-1.26
CML442xCZL04006	8.25	3.41	265.61	3.18	0.63	304.3	2.24	0.22	-0.49
CML312xCML444	12.4	3.36	338.88	3.96	1.41	591.2	2.09	0.21	1.71
CML502xCZL1	9.54	4.13	188.33	2.32	0.41	613.4	2.01	-0.62	0.02
CML395xCZL04006	9.78	3.41	257.79	3.58	0.77	367.5	1.94	0.26	-0.49
CML312SRxCZL068	9.49	4.29	186.07	2.62	2.03	146.7	1.84	-1.26	1.24
CML444xCZL2	10.34	3.36	278.26	2.31	1.41	363.7	1.64	1.71	-1.33
312SRxCZL04006	8.24	4.29	137.72	3.71	0.87	327.2	1.51	-1.26	-0.49
CML442xCZL2	6.96	2.96	263.76	2.54	0.70	340.8	1.42	0.22	-1.33
CML312xCZL2	9.57	2.25	372.81	1.32	0.70	126.6	1.31	0.21	-1.33
CML312xCML502	7.47	1.95	373.52	2.65	0.25	591.2	-0.82	0.21	-0.62
CML395xCML502	7.02	2.61	255.59	3.05	0.77	162.2	-0.88	0.26	-0.62
CZL1xCZL04006	6.74	4.13	118.25	2.65	0.63	166.5	-0.99	0.02	-0.49
CML442xCML502	6.72	2.96	207.06	1.85	0.47	74.5	-1.01	0.22	-0.62
CML395xCML444	9.47	3.36	240.02	4.25	1.41	314.6	-1.21	0.26	1.71
CML442xCZL068	8.51	4.11	155.79	3.54	2.03	156.2	-1.42	0.22	1.24
CML312xCZL04006	6.25	3.41	152.01	3.81	0.63	348.8	-1.61	0.21	-0.49
CML442xCZL1	6.96	4.13	113.68	3.02	0.47	556.3	-1.89	0.22	0.02
CML444xCZL04006	6.84	3.41	158.75	4.08	0.63	383.4	-2.87	1.71	-0.49
CZL2xCML312SR	9.61	4.29	-76.15	3.52	0.87	-66.6	-6.85	-1.33	-1.26
MEAN	7.91	3.14		2.59	1.46		0.00	0.00	0.00
LSD	2.11			1.89			1.16	0.62	0.62

Key - GY: Grain yield; HPYld: High parent yield; AD: Anthesis date; % Het: % Heterosis

Table 4.6 Grain yield, Heterosis, SCA effects and GCA effects for yield of 20 selected hybrids (the best 10 and the worst 10 based on SCA effects for yield).

Whilst hybrids in which CML502 was involved gave high heterosis percentages, the line had a negative GCA estimate for yield and had low SCA estimates in the crosses where it was involved. Other crosses that had negative SCA estimates had very high heterosis estimates. Of particular interest was the single cross tester for group B (CML395 x CML444), which had a negative SCA estimate (-1.21) as was expected, but high heterosis (240% for optimum and 314% for low N) was realized after crossing the two (Table 4.6).

CHAPTER5

DISCUSSION

5.1 Grain yield and its components under low N (CIMMYT-Harare)

Grain yields for the single cross hybrids under low N were on average 2.98t/ha (Table 4.2) and for the inbred lines was 0.62t/ha under these conditions (Appendix A). Compared to the optimal environment trials, the single cross hybrids yielded on average 34% of the yield obtained under optimal conditions while the inbred parental lines yielded 20.1%. These averages were within the stipulated limits recommended by Bandier et al. (1997) who recommend that N deficiency should reduce yields by greater than 40% in order for mechanisms of tolerance to be brought into into play. They also added that within these limits, N stress will be sufficient enough to expose genotypic variation for tolerance to low N. Bolanos and Edmeades (1996)also determined that average yields under such abiotic stresses should be between 20% and 30% of what the average yield would have been in the same location under optimum management to be able to select varieties that perform reasonably well both under stress and optimum conditions. In contrast, the average yields of the inbred lines were very low (0.62t/ha) indicating that the effect of the stress was more severe on the inbred lines than on the hybrids.

This was in agreement with Betran *et al.* (2003) who reported that parental lines were relatively more sensitive to low N stress than the hybrids formed from those lines. Thus the results indicated better adaptation of the hybrids to low N stress and consequently, high heterosis estimates were observed under low N conditions. Although some of the single cross hybrids from the new elite lines out-yielded the hybrids from the currently used inbred lines, (CML312/CML442 and CML395/CML44) under low N stress conditions (Appendix D), most of the new parental lines had negative GCA effects for yield (Figure 4.1). Grain yield and its components (EPP and 1000KW) were positively associated. Hybrids had the same relative performance for grain yield and EPP whilst there were some differences with 1000KW.

5.1.1 Secondary traits under low N stress

In the present study, there was a consistent relationship between ASI and grain yield where by single cross hybrids that had a lower value for ASI had high grain yields (Appendix D). ASI was negative for 3 hybrids only, CML442/CML44, CML312/CML395 and CML502/CZL1, and the yields were 4.17t/ha, 3.87t/ha and 2.68 respectively (Appendix A). All the yields were above the trial mean yield of 2.54t/ha, which indicated that a shorter ASI translated to higher yields. Similarly, hybrids that had the highest values for ASI also were among the poorest performing for yield. CZL2/CML312SR, CML395/CZL068, CML395/CZL1 and CZL2/CZL04006 had high and positive values for ASI, which are as follows 20.7days, 6.4 days, 5.9 days and 4.8 days respectively (Appendix D). The corresponding yields for these hybrids were 0.34t/ha, 1.56t/ha, 2.31t/ha and 2.21t/ha respectively and were below the trial have mean yield (Appendix A).

This was related to what was established by Bolanos and Edmeades (1992) that ASI is an indicator of assimilate partitioning to the growing ear at flowering rather than variability in plant water status or nutrient status. A greater ASI is associated with drought and low soil fertility susceptibility, slow ear growth, barrenness and low harvest index whilst a shorter ASI could be equated to fewer but larger florets that grow more rapidly at anthesis and which are therefore more tolerant of reductions in photosynthesis caused by drought or other stresses (Westgate, 1994).

Most of the single cross hybrids (Appendix D) and inbred lines (Appendix A) that had low leaf senescence values also had high grain yields. CML444 and CZL068 were the only lines that had yields above 1t/ha under low N stress (Appendix A) and their leaf senescence scores were 0.8 and 0.5 respectively (Appendix A). Low N stress accelerates leaf senescence, and genotypes with good nutrient partitioning have been shown to delay senescence (Banziger et al., 1997). Premature leaf senescence is a result of insufficient transpirational cooling and the subsequent heating of all or parts of the leaf to lethal temperatures (Blum, 1988). Fischer *et al.* (1983) found that for a selection programme in

maize, the rate of leaf senescence under stress across various selections was negatively correlated with yield under stress, ($r = -0.48^{**}$); hence lower values for senescence are favorable.

There was no consistent relationship between hybrid or inbred line chlorophyll concentration and grain yield or leaf senescence score. The expectation was to have higher yields in those hybrids that had more chlorophyll concentration and a lower score for senescence. CZL068, which was the best performer for yield, did not have the highest chlorophyll content but had a low score for senescence, which has been seen to have contributed to the high yield. The inconsistency observed in these trials, and the apparent lack of progress in selecting for this trait in Tuxpeno Sequia (Bolanos and Edmeades 1993b), may indicate that increased demands for N by the larger ear resulting from selection need to be met by mobilization of N from the leaves, thereby reducing the amount of chlorophyll in the leaves (Muchow, 1994).

5.2 Grain yields under optimal environment (Art Farm)

The significant differences that were observed amongst the entries for grain yield (Table 4.1) showed that the hybrids were significantly different from each other and hence the best performing single cross testers can be identified. Hybrids that were formed from parents with the high and positive GCAs (CML444 and CZL068) also had high yields (Appendix C). Under the same growing conditions, the parental lines also showed differences in their performances and the best performing parents managed to pass on favorable genes for grain yield to their offspring.

Husk cover (HC) and grain texture (TEX) were only measured at this site only because husk-covering problems do not manifest well on cobs grown under stress as these will be poorly filled. Similarly grain texture was also observed under the optimum site only because the expression of the trait is severely distorted under stress. Both the inbred lines and the parents showed much variability for these two traits as shown by significant mean squares (Table 4.1). While there was a tendency for high yielding inbred and the corresponding hybrids formed to have husk-covering problems, this was not the case for

CML444 and CZL068. The problem was very pronounced in hybrids in which CML312SR was involved even though the inbred line did not have a high husk cover percentage. The value was 4.1 (Appendix B). These two traits, (HC and TEX) and other traits that were measured at one site only were not included in the selection for new testers since for them, information on genotype by environment interaction is lacking in addition they are qualitative traits which are not necessarily related to yield and its components.. Scott (1967) reported that G x E interaction is helpful in selecting stable genotypes (genotypes that exhibit the least trait variation across environments).

ASI was not significant under optimal conditions (Table 4.1) in comparison to the low N environment. The mean value for ASI was 3 days (Table 4.2) under low N conditions and was 0.5 days under optimal conditions (Table 4.1). This was in line with the findings by Banziger and Lafitte (1993) that under optimal conditions, silks emerge round the same time that pollen is shed and genotypes do not show much variability for this trait and the values obtained will be small and close to zero. However, under stress, ASI is lengthened and genotypes with stress tolerance will have a shorter ASI.

5.3 Across site analysis

For this study, it was necessary to know if G x E interactions were significant so that selections will be targeted at the stable genotypes – genotypes which will not give a huge yield penalty if grown under stress environments. The significance of variation to environments for all the traits except for EPO (Table 4.3) confirmed the two sites to be uniquely different from each other as was expected. Similarly, hybrids showed substantial environmental and genotypic variability across environments.

Significant interactions of entries for GY, AD, ASI, EPP, RL, TEX, CHL, and 1000KW with the environments meant that genotypes that performed well at the optimum environment were not necessarily the best yielders at the low N environment for these traits (that is performance changed with environment). However, some crosses performed well across the two environments. The top 5 yielders under optimal conditions were CML312/CML444 (13.35t/ha), CML444/CZL068 (12.65t/ha), CML312SR/CZL068

(12.43t/ha), CML444/CML312SR (11.95t/ha) and CML395/CML312SR (11.7t/ha)(Appendix C). For the low N trial, the top 5 yielders were CML395/CML312SR (5.63t/ha), CML444/CZL068 (5.45t/ha), CML444/CML312SR (4.97t/ha), CML312SR/CZL04006 (4.62t/ha) and CML395/312SR (4.15t/ha). This was in agreement to the findings by Betran *et al.* (2003) that with hybrids that perform well across environments it is possible to combine stress tolerance and yield potential in tropical maize. Similar results have been reported with temperate maize hybrids where improvements for tolerance to abiotic and biotic stresses have been associated with the ability to maximize grain yield under non-stress growing conditions (Duvick, 1997).

Single crosses are sensitive to environments (Hallauer *et al.*, 1988); hence it was not surprising that all traits interacted significantly with the environments in this study. The fact that single crosses are sensitive to environments is also the reason why breeders are targeting to produce three way hybrids for marginal environments, which are stable and have a broader genetic base. In addition to single crosses being sensitive to environments, stress environments produce high genotype by environment interactions (Banziger *et al.*, 2000). Yield and plant height generally decrease under stress: Mean grain yield for hybrids was 8.36t/ha (Table 4.1) under optimal conditions and 2.54t/ha under low N stress (Table 4.2) while mean plant height was 251cm (Table 4.1) under optimal conditions and 170.4 under low N (Table 4.2). The anthesis-silking interval is known to increase under stress and has been one of the secondary traits that have been used successfully to improve selection efficiency under stress(Bolanos and Edmeades, 1993a).

5.4 General Combining Ability (GCA) effects for grain yield

The inbred lines CML444 and CZL068 had the highest yields (1.63t/ha and 1.03t/ha respectively) under low N conditions and also the highest GCA effect estimates for grain yield among the lines tested (Figure 4.1). On the contrary, CML312SR, which had the highest yield under optimal conditions (Appendix B), had negative GCA effects for yield (Figure 4.1). This indicated a considerable relationship between *per se* performance of the inbred line and GCA effects which might be the main cause for variability in GCAs

among inbred lines. Similar trends in GCA effects for yield were observed among normal maize lines (Banziger *et al.*, 2002) and among drought tolerant lines under well-watered conditions and stress conditions in Mexico (Betran *et al.*, 2003).

However, the single cross CML312SR/CZL2 could explain the negative GCA effects for CML312SR. This cross gave low yields at both environments and had the least SCA effect (-6.89) for grain yield (Table 4.5). Both lines are in designated heterotic group A (Table 3.1). Consequently, the cross had negative heterosis estimates (Appendix E). The early maturing line CML502 had the least estimate for grain yield GCA effects (Figure 4.1) as compared to the late CML444 and CZL068 which were superior in yield performance and GCA estimates. The trend was similar for yield performance. This was in agreement with the observation by Vivek *et al.* (2002) that maturity and yield are positively correlated as it is quite obvious that a late maturing variety has the opportunity to draw nutrients and photosynthesize over a longer period while an earlier maturing variety is predisposed to lower yields owing to its shorter life cycle. In contrast, under stress an early maturing variety can complete its life cycle early and escape stress.

The GCA estimates ranged from -1.3(CML502) to 1.7(CML444) under optimal conditions and from -0.7(CML502) to 0.6(CML444) under low N conditions (Figure 4.1). The results indicate that GCA effects of the parental lines were variable in both magnitude and direction and in sensitivity across the two environments. Significantly positive GCA effects for grain yield are essential for maize genotypes to sustain production under stressed and non-stressed conditions. The highly significant GCA effects for grain yield implied that the inbred lines contributed differently in the crosses in which they were involved. These results suggested adequate diversity in the genetic expression of parents for yield under the two contrasting environments. CML444 and CZL068 were good general combiners for yield at both environments and this indicated that the lines managed to confer favorable genes for yield in all their crosses or indicated effective transmission of favorable genes from these two parents.

The significant differences of GCA mean squares for grain yield showed that variability in grain yield was being controlled by additive genes making identification of testers based on GCA for grain yield possible. Inbred line testers are selected on the basis of GCA effects as this is highly heritable while hybrids are selected on the basis of SCA effects (Hallauer and Miranda, 1988). The highest priority is given to yield although its genetic variability is reduced under stress.

5.4 GCA effects for other agronomic traits and secondary traits

There were significant differences for GCA effects estimates for the other traits that are secondary to the primary trait of interest (grain yield).

5.5.1 Grain yield components

Four of the parental lines (CML312, CML444, CZL068 and CZL04006)(Table 4.4) consistently showed positive GCA effects for grain yield components (EPP and 1000kw), which was an indicator of promising, yield performance (Table 4.5). CML395 had the highest positive GCA estimate for 1000kw but had a negative GCA effect for EPP (Table 4.4). The positive GCA effect (Figure 4.1) observed in yield for CML395 appeared to be the consequence of positive GCA effects for 1000kw only since it had a significantly negative GCA for EPP(Table 4.4) . Similar studies considered low prolificacy as a major factor-limiting yield under stress conditions while positive GCA estimates for EPP is a good indicator of stress tolerance (Banziger and Lafitte, 1999). EPP is an important secondary trait that is used to select drought and low N tolerant germplasm.

5.5.2 Secondary traits

There was considerable consistency in the inbred lines results with GCAs, SEN and CHL. Lines that had negative GCAs for leaf senescence (Table 4.4)had high and positive GCA estimates for leaf chlorophyll content as well as grain yield (Table 4.4). However, this did not always translate to higher yields for the inbred lines although lines that had negative GCA estimates for senescence always performed better for grain yield than

those with positive values. For leaf senescence, positive GCA values are an indication of rapid senescence while negative values indicate reduced senescence. When selecting for abiotic stress tolerance, it is genotypes that senesce slowly or stay green for a longer period of time that are mostly preferred (Banziger and Lafitte, 1997).

Inbred lines that were late maturing and had a shorter ASI always had positive GCA estimates for grain yield (Table 4.4) although the magnitude varied widely. Positive GCA effects for yield are associated with lateness and ironically more yield. A shorter ASI directly translates to more yield because there will be less barren ears; in addition a shorter ASI is also associated with increased partitioning of assimilation to the developing ear at the reproductive phase (Edmeades *et al.*, 1993)

5.5.3 Agronomic traits

Short statured inbred lines had negative GCAs for PH and the corresponding EH (Table 4.4). For PH, half of the lines (CML312, CML395, CML444, CML502, CZL1 and CZL04006) had positive GCAs and the other half had negative (Table 4.4). In general, of the five lines that were good general combiners for PH (negative GCAs) only 2 of them responded well in terms of yield and stress adaptive traits (Table 4.4). CZL068 and CZL04006 had significant negative effects for AD, PH and EH, and this indicated them as good general combining parents for these traits as they managed to have a reduced height and possess genes for earliness. Whilst CZL2 had a negative GCA estimate for ASI, PH, EH and EPO (Table 4.4), it may not be superior to the conventional lines because it had a negative GCA estimate for yield (Figure 4.1). Hence it is clear that for this set of inbred lines, the tall and late maturing lines were superior in *per se* yield performance and in GCA estimates for yield.

For grain texture, negative GCA estimates are preferred as small-holder farmers and consumers in most of southern Africa prefer flint maize (CIMMYT, 2001). CML442, one of the conventional lines had good GCA for yield but the line is very dent and had the highest positive GCA estimate for TEX(1.26)(Table 4.4). Early maturing lines had negative GCA estimates for MOI while the reverse was true for late maturing lines.

5.6 SCA effects for grain yield under optimal conditions

The SCA estimates for the single crosses ranged from -6.82 (CZL2/CML312SR) to 2.69 (CML395/CML312SR) (Table 4.5). A cross that had a positive SCA effect mostly implies that the inbred lines constituting that cross were genetically divergent among themselves and belong to different heterotic groups. Betran *et al.* (2003) found out that cross combinations from diverse genetic backgrounds had positive SCA effects with high performance while the reverse held true for closely related parents. The farther the value is from zero, the more distantly related the lines are while the closer to zero the value is, the more closely related they are. From previous classification (CIMMYT, 2001), it has been established that CML312 and CML442 are in-group A (hence constitute the heterotic group A tester) while CML395 and CML444 are from heterotic group B. Based on these classifications, the cross between CML312 and CML442 was expected to have a negative value and similarly for CML395 and CML444 (Table 4.5). In the same context, crossing CML312 to any of the two group B lines was supposed to give a positive value. This was the case for three of the crosses but not for the cross between CML395 and CML312 whose SCA estimate was negative (-2.06)(Table 4.5) yet they are from opposite heterotic groups. There were six other crosses that had such a phenomenon and this inconsistency made it difficult to determine the genetic relationships between parents.

Such seeming changes in heterotic behavior are not unusual as combining abilities are specific to the group of parents being tested. Also, lines belonging to the same heterotic group may not have absolutely identical heterotic patterns because of small differences in the alleles they may be carrying (Rawlings and Thompson, 1962). Hallauer and Miranda (1988) reported that heterotic groups are not absolute and thus a changing or reclassification of lines into heterotic groups may be necessary. Even though one would expect negative SCA effects for crosses from lines in the same heterotic group, sufficient variation exists within heterotic groups of CIMMYT germplasm indicating the groups could be further refined. Materials defined as being in heterotic group AB (CML502) for the CIMMYT Zimbabwe breeding program, have high heterosis with both groups A and B.

For a breeding program geared towards development of 3-way hybrids and double cross hybrids, this presents more options where hybrids could be developed using all three heterotic groups for example an A x B single cross will be crossed to a line from AB heterotic group without having to deal with the inbreeding depression of the female seed parent that one might encounter when an intra group cross is made to form the seed parent.

The largest SCA effects (> 2) for grain yield were contributed by CML395/CML312SR(2.69), CML442/CZL04006(2.24), CML312/CML444(2.09) and CML502/CZL1(2.01) across the two sites (Table 4.5). These crosses were also superior in yield performance at both environments except for CML502/CZL1, which performed poorly at both environments although its heterosis was relatively high (Appendix E). It is therefore demonstrated that high yielding crosses showed high SCA values indicating the importance of SCA effects in predicting F₁ hybrid performance for grain yield. This observation was consistent with a similar study in Mexico, which suggested that SCA predicts hybrid yield better than heterosis, since it is not affected by the parental performance (Betran *et al.*, 2003). Consistent negative SCA effects accompanied with poor performance in each of the individual environments and across environments were observed for CML395/CZL068, CML395/CML502, CZL2/CML312SR, CML502/CML312SR and CML502/CZL04006 (Appendix C).

5.7 Grain yield, SCA and heterosis

The average degree of high parent heterosis per environment varied from -66% (CML312SR/CZL2) to 883% (CML312/444) at the low N environment (Appendix E) and -76% (CML312SR/CZL2) to 373.5% (CML312/CML502) at the optimal environment (Appendix F). The experiments under low N had extremely high expression of heterosis because of the relatively low performance of the inbred lines under stress. There seemed to be a positive relationship between hybrid grain yield and the corresponding SCA effect and high parent heterosis (HPH) estimates in each environment although no test was conducted to test the significance and magnitude of the relationship. There was intra heterotic group HPH with the conventional tester lines

having positive SCAs and high heterosis when crossed to lines from within the same heterotic group. The study indicated heterosis to be an important factor in increasing maize yields and on the average, F₁ showed 250% higher yields than the mean of the parents under optimal conditions and 450% under low N conditions (Appendix E). Heterotic response was likely to be high because of the divergence of the parental material as well as diversity within each heterotic group.

The primary criterion for choosing parents that might have high heterotic response and subsequently produce superior yielding F₁ would be the GCA effect as suggested by Hallauer and Miranda, 1988. A positive heterosis estimate satisfies one of the important criterion for single cross testers, which states that they should not suffer much inbreeding depression (Sprague and Tatum, 1942).

In general, there was consistency between grain yield and SCA, that is where SCA effect was high and positive, the corresponding hybrids had a superior performance for yield. However, the trend was not observed in both environments. The hybrids exhibiting the highest heterosis ultimately had high yields although there was no consistency in rankings between the two environments (Appendix E and Appendix F). This is an indication that the GCA among parental lines can predict hybrid performance better than the heterosis observed which is highly dependant on the performance of inbred lines. Furthermore, as a consequence of the differential response of the inbred lines to stressed environmental conditions relative to the hybrids, heterosis was more erratic and inconsistent across environments than SCA.

Heterosis *per se* will not determine the performance of any hybrid; because it has been observed that some better yielding hybrids may not necessarily exhibit high heterosis compared to hybrids with very high heterosis, but poorer yields. This is because yield is not determined by heterosis only, but there are other genetic contributions which play a role e.g. additivity (Duvick, 1984) and that heterosis is a function of the inbreeding coefficient (the more the line is inbred, the more heterosis it might show in the crosses)(Falconer, 1960). Parents for single cross hybrids sometimes share some common

ancestors in their pedigrees. This has been the reason breeders have managed to exploit heterosis fully by crossing distantly related material. Ideally single crosses to be used as seed parent testers should come from parents of the same heterotic group and still exhibit heterosis (Vivek *et al.*, 2004) but at the same time, the primary criteria for choosing parents that might have high heterotic response and subsequently produce superior yielding F₁ would be the GCA effect of the parents.

CHAPETR 6

CONCLUSIONS

6.1 Conclusion

Two promising single cross testers were identified for the two heterotic groups at CIMMYT and the single cross hybrids are CML312/CZL04006 for heterotic group A and CML444 for heterotic group B.

6.1.1 Tester identification

All the lines (CML312, CML395, CML442 and CML444) currently being used in single cross testers were still among the best and managed to have positive GCAs for grain yield and the components of grain yield. CML444 emerged to be the best line based on GCA estimates and from the findings of this study, the line is difficult to replace. The choice of potential tester was based primarily on display of positive GCA effects at the two contrasting environments. Other factors that were taken into consideration to aid identification were high GCA estimates for secondary traits (EPP, SEN ASI and CHL), heterosis estimates, grouping of the inbred lines constituting the single cross into the same heterotic group and *per se* grain yield.

Based on this, only two lines (CZL068 and CZL04006) managed to show potential to replace CML395 and CML442 respectively. The single cross CML312/CZL04006 was identified as the potential tester for group A with CZL04006 replacing CML442. While CML442 had high and positive GCA estimates for grain yield at the optimal environment, it had a negative GCA estimate for the low N environment, which meant that the line did not display much stress tolerance in the crosses where it was involved. The line is also heavily dent as compared to CZL04006, which is semi flint and considering the preferences of the targeted people; a flint line would be preferable.

For heterotic group B, the single cross CML444/CZL068 was identified as a potential tester with the cross being constituted of the parents that had the highest positive GCA estimates for yield. Both lines are late maturing and have semi-flint grain. The single cross was the best performer for yield under low N conditions and ranked among the best when an across site analysis was done indicating a high degree of stress tolerance. The two lines involved in the single cross also had high and positive estimates for EPP and 1000kw as well as for CHL while they had negative estimates for ASI and SEN.

6.2 Recommendations

- a) As a further step, verifying the utility of the promising testers in early generation testing of lines needs to be conducted in order to give more information on the utility of the proposed two new testers and relate their performance to the current testers.
- b) Also as a further step, additional evaluation of the hybrids under drought and other locations in order to get more information on G x E interactions needs to be done.
- c) DNA fingerprinting would be useful for refining the heterotic groups of the lines used in this study.
- d) The single cross data obtained from this study should be used to decide on new pedigree breeding and in the predicting of performance of new three-way and double cross hybrids.

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APPENDICES

APPENDIX A: Grain yield and its components for inbred parental lines under low N conditions

Line	Pedigree	GY	Rank	AD	ASI	PH	EH	EPO	EPP	SL	SEN	KW	CHL
CML312	CML312	0.25	11	81.4	12.4	112.0	33.7	0.29	0.24	0.7	1.0	191.3	44.4
CML395	CML395-B	0.77	3	72.0	4.8	109.5	33.6	0.33	0.77	6.6	1.5	138.8	41.4
CML442	CML442	0.47	8	80.1	11.1	96.6	29.2	0.31	0.71	-1.0	4.5	141.3	47.8
CML444	CML444-B	1.63	9	84.2	4.8	82.4	38.1	0.46	0.55	0.4	0.8	143.5	43.1
CML502	CML502	0.21	12	82.4	10.0	100.6	35.0	0.34	0.43	0.9	2.0	85.9	50.2
CZL1	[CML442/CML197//[TUXPSEQ]C1F2/P49-SR]F2-45-7-3-2-BBB]-2-1-1-1-1-B*4-B	0.32	10	82.7	20.1	102.2	37.2	0.36	0.45	1.8	0.9	161.8	29.8
CZL2	[(CML395/CML444)-B-4-1-3-1-B/CML395//DTPWC8F31-1-1-2-2]-5-1-2-2-BB-B	0.70	4	76.8	16.5	72.1	30.7	0.47	0.78	7.1	0.9	134.8	38.8
CML312SR	MAS[MSR/312]-117-2-2-1-B*4-B-B	0.87	2	72.3	2.3	107.8	38.9	0.35	0.87	0.3	1.0	128.1	36.5
CZL068	[LZ956441/LZ966205]-B-3-4-4-B-5-BBBBB-B	1.03	1	79.6	1.2	88.6	33.0	0.36	0.81	0.8	0.5	182.7	32.7
CZL04006	ZM621A-10-1-1-1-2-BBBBBB-B-B	0.63	6	81.4	0.5	91.2	35.7	0.37	1.04	0.7	0.7	122.1	36.2
Mean		0.87	7	78.8	9.3	92	33.8	0.32	0.65	1.5	1	149.6	40.2
LSD						40.3	17.8		0.44	6.6	0.1	50.2	10.8
MSe		0.00	4	18.6	0.0	0.1	0.0	0.00	0.1	0.0	0.05	0.1	0.0
CV		0.62		5.5	1.4	0.3	0.5	0.3	49.71	3.5	1.23	0.2	0.2
p		0.45		0.12	0.012	0.347	0.719		0.09	0.317	0.0014	0.002	0.043

APPENDIX B: Grain yield and its components for inbred parental lines under optimal conditions.

		GY	Rank	AD	ASI	PH	EH	MOI	HC	EPP	CHL
CML312	CML312	3.43	11	160.5	-0.5	156.4	70.9	16.6	8.1	0.80	50.6
CML395	CML395-B	2.95	7	156.6	1.3	131.4	59.2	14.9	10.0	0.91	27.8
CML442	CML442	3.09	6	158.9	-0.6	158.3	74.4	15.4	9.1	1.09	40.7
CML44	CML444-B	3.69	5	160.8	-0.1	156.5	89.4	13.9	6.7	1.05	51.9
CML502	CML502	0.95	14	162.5	4.5	139.7	68.2	13.5	4.3	0.39	31.4
CZL1	[CML442/CML197//([TUXPSEQ]C1F2/P49-SR)F2-45-7-3-2-BBB]-2-1-1-1-1-B*4-B	4.22	3	161.2	0.3	146.9	72.1	17.3	6.9	1.17	38.5
CZL2	[(CML395/CML444)-B-4-1-3-1-B/CML395//DTPWC8F31-1-1-2-2]-5-1-2-2-BB-B	2.45	10	157.4	2.3	131.4	81.3	16.2	8.0	0.95	43.4
312SR	MAS[MSR/312]-117-2-2-1-B*4-B-B	3.51	1	153.1	0.6	141.8	64.0	16.4	4.9	1.19	32.3
CZL068	[LZ956441/LZ966205]-B-3-4-4-B-5-BBBBB-B	4.37	2	155.0	-0.3	137.9	65.0	13.6	9.0	1.12	43.1
CZL04006	ZM621A-10-1-1-1-2-BBBBBB-B-B	4.51	8	157.4	0.0	154.6	72.2	13.8	6.7	0.91	48.7
								14.0			48.2
	Mean	2.98	8	157.6	1.2	140.6	71.0	13.1	7.4	0.92	42.3
	LSD		4	7.0	5.4	21.2		0.21	4.6		0.02
	MSe	5.43		0.0	0.0	0.0	177.0	4.5	0.0	0.00	9.2
	CV	78.3		0.0	3.2	0.1	18.7	0.021	0.5	0.33	0.002
	p	4		0.123	0.385	0.004			0.210		

APPENDIX C Grain yield and its components for single cross hybrids at ART farm under optimum conditions.

Entry	Pedigree	GY T/ha	AD days	ASI days	PH cm	EH cm	EPO cm	EPP 0-1	MOI %	CHL spads	HC %	ET 0-5	1000KW grams
1	CML312/CML395	8.882	72.5	0	257.5	140	0.517	1.00	19.15	62.62	16.540	1.5	418
2	CML312/CML442	11.277	74	0.5	257.5	136	0.557	0.97	20.05	62.92	8.824	2	377
3	CML312/CML444	13.356	76	0	267	153	0.577	1.47	20.20	59.81	13.889	1.5	374
4	CML312/CML502	9.279	73	1.5	263.5	130.5	0.488	1.57	15.90	60.13	7.738	1.5	276
5	CML312/CZL1	11.713	73.5	-1	267	132.5	0.525	1.46	20.55	62.07	5.719	1.5	327
6	CML312/CZL2	10.665	70	2.5	248.5	122	0.513	0.97	19.60	60.95	11.111	1	347
7	CML312/CML312SR	9.390	70	-1	251.5	110	0.489	1.02	18.50	66.12	11.455	1.5	362
8	CML312/CZL068	11.660	69	-0.5	258	130	0.518	1.18	18.65	63.46	12.385	1.5	424
9	CML312/CZL04006	8.582	71	-1.5	256.5	130	0.529	1.04	18.70	60.34	8.333	1.5	386
10	CML395/CML442	11.074	73.5	3	259	148.5	0.575	1.00	19.90	56.03	5.556	2	365
11	CML395/CML444	11.226	77	-1	266	159	0.562	1.00	18.80	52.51	5.556	1.5	380
12	CML395/CML502	9.262	74.5	1	271	150	0.561	0.97	16.25	53.34	5.556	2	380
13	CML395CZL1	10.507	74	0	267	125.5	0.498	0.97	20.65	55.89	5.556	1.5	389
14	CML395CZL2	9.400	72.5	18	260	135	0.554	0.80	20.00	53.14	6.552	1.5	363
15	CML395CML312SR	11.748	70.5	1.5	252.5	121	0.494	1.03	19.45	57.39	11.111	1.5	405
16	CML395/CZL068	11.646	74	1	247	144	0.574	1.00	22.35	55.34	5.556	1.5	446
17	CML395/CZL04006	12.184	73	1.5	263	154.5	0.620	0.97	15.25	53.46	5.556	2	371
18	CML442/CML444	11.998	75	-0.5	266.5	160	0.622	1.08	18.60	59.8	8.333	1.5	322
19	CML442/CML502	9.109	77	0	253.5	138	0.571	1.17	15.95	55.19	5.719	2.5	238
20	CML442/CZL1	8.830	77	0	254	127.5	0.472	0.97	21.90	57.35	5.556	2	378
21	CML442/CZL2	10.790	69.5	5	255.5	124	0.492	0.97	21.20	59.84	8.333	1.5	375
22	CML442/CML312SR	10.018	69	0.5	244	114	0.472	0.94	19.20	60.75	8.333	2	303
23	CML442/CZL068	10.517	70.5	0.5	232	132.5	0.570	0.97	16.60	60.4	14.379	1.5	336
24	CML442/CZL04006	12.450	74	0	255	132	0.534	0.95	17.40	56.58	4.144	2.5	309
25	CML444/CML502	12.266	76.5	0	278	167.5	0.596	1.22	17.90	54.55	5.556	1.5	277
26	CML444/CZL1	11.922	79	-3	284	150.5	0.509	1.69	17.90	56.91	5.556	1.5	339
27	CML444/CZL2	12.488	76	3.5	265.5	150	0.540	1.14	20.40	54.72	8.497	1.5	361
28	CML444/CML312SR	11.954	72	-1.5	256.5	130.5	0.526	0.92	18.20	61.16	5.556	1.5	397
29	CML444/CZL068	12.657	73	-0.5	253.5	162	0.575	1.00	20.20	58.45	5.556	1.5	392
30	CML444/CZL04006	8.811	77	-3.5	266	154	0.590	1.06	17.60	53.76	8.333	2	361
31	CML502/CZL1	11.915	76	2.5	259	124	0.558	1.80	19.15	52.92	5.556	1.5	265
32	CML502/CZL2	9.359	73	1.5	248	136.5	0.535	0.94	19.55	53.62	8.333	1.5	275
33	CML502/CML312SR	7.859	72	-0.5	238.5	111.5	0.463	0.92	18.20	63.04	11.869	1.5	302
34	CML502/CZL068	10.887	71.5	1.5	253.5	131.5	0.543	1.19	18.05	56.51	16.667	2	326
35	CML502/CZL04006	9.661	74.5	0	263.5	137	0.535	1.11	17.85	55.15	8.333	2	302
36	CZL1/CZL2	9.236	71	1.5	260.5	122.5	0.500	0.94	22.05	59.09	11.111	1.5	355
37	CZL1/CML312SR	9.870	71	0	243	128	0.568	1.06	20.30	59.81	6.349	1.5	388
38	CZL1/CZL068	11.400	71.5	1	250.5	136	0.534	1.06	19.35	60.08	5.903	1.5	431
39	CZL1/CZL04006	9.019	76	0	255	122.5	0.466	1.14	20.15	53.72	5.556	1.5	330

Entry	Pedigree	GY	AD	ASI	PH	EH	EPO	EPP	MOI	CHL	HC	ET	1000KW
40	CZL2/CML312SR	1.025	78	2.5	198	83.5	0.592	0.47	16.35	35.67	5.556	1.5	114
41	CZL2/CZL068	11.827	66.5	0	188	125.5	0.504	1.00	16.55	62.2	8.333	1.5	381
42	CZL2/CZL04006	8.797	72	0.5	242	133	0.506	0.92	19.50	56.16	5.556	1.5	315
43	CML312SR/CZL068	12.432	67.5	-0.5	241	108	0.502	1.00	16.10	62.23	8.333	1.5	373
44	CML312SR/CZL04006	10.218	71	-2	241.5	112.5	0.410	1.00	18.05	61.59	8.333	2	367
45	CZL068/CZL04006	10.626	71.5	-1.5	243	113	0.449	1.00	16.75	56.32	5.556	2	376
Mean		8.83	71.2	0.5	251	133	0.52	1.04	16.23	73.66	8.33	1.5	326
LSD		0.62	0.75	1.73	3.23	4.5	0.017	0.05	0.83	0.92	1.21	0.14	13.98
Mse		0.69	0.47	1.69	2.56	7.6	0.0025	0.084	0.62	0.45	0.98	0.24	8.69
p		0.0025	0.0598	0.879	0.0258	0.0784	0.987	0.0069	0.0095	0.004	0.008	0.048	0.0057

APPENDIX D: Grain yield and its components for single cross hybrids at CIMMYT Zimbabwe under low N conditions.

Entry	Pedigree	GY T/ha	AD days	ASI days	PH cm	EH cm	EPO cm	EPP 0-1	MOI %	CHL Spad units	KW grams	SEN 1-10
1	CML312/CML395	3.378	73.5	-0.5	215	105	0.455	1.100	10.5	44.48	295.80	4.3
2	CML312/CML442	3.086	74	1.5	185	85	0.474	0.786	10.6	39.35	246.65	4.0
3	CML312/CML444	4.832	78	2.5	227.5	125	0.622	0.808	11.4	34.34	264.10	4.3
4	CML312/CML502	1.728	77.5	1.5	205	102.5	0.500	0.858	10.7	33.40	191.00	4.5
5	CML312/CZL1	2.255	77.5	1	190	82.5	0.436	0.631	12.5	28.35	260.30	4.5
6	CML312/CZL2	1.292	74	4	177.5	85	0.457	0.714	10.6	27.48	237.00	4.5
7	CML312/CML312SR	3.190	73	4	182.5	90	0.496	0.878	11.43	39.70	262.85	4.9
8	CML312/CZL068	3.470	72	2	190	90	0.464	0.858	12.65	40.72	294.58	4.0
9	CML312/CZL04006	2.828	73	2	187.5	92.5	0.500	0.974	11.3	38.66	268.75	4.5
10	CML395/CML442	2.593	75.5	4	172.5	85	0.500	0.918	10.5	32.11	228.20	5.0
11	CML395/CML444	3.193	80	1	182.5	95	0.486	0.824	11.2	30.00	266.00	4.0
12	CML395/CML502	2.019	77.5	1.5	182.5	95	0.485	0.906	10.5	28.11	195.65	5.0
13	CML395CZL1	1.954	77.5	6	192.5	92.5	0.462	0.867	10.6	22.25	234.60	4.3
14	CML395CZL2	2.535	76	6.5	175	85	0.498	0.927	10.98	25.62	226.77	5.0
15	CML395CML312SR	4.156	72.5	4	182.5	92.5	0.474	1.187	10.5	36.21	249.70	4.8
16	CML395/CZL068	5.123	74.5	6.5	165	75	0.471	0.746	10.7	28.54	264.75	5.3
17	CML395/CZL04006	3.600	77	1	195	100	0.524	0.913	11.6	33.88	255.95	4.0
18	CML442/CML444	2.510	78	-0.5	167.5	90	0.485	0.950	10.5	27.37	223.45	4.3
19	CML442/CML502	0.820	76.5	4.5	167.5	70	0.389	0.632	10.6	23.68	175.25	6.0
20	CML442/CZL1	3.085	78	2.5	180	85	0.444	0.875	10.6	33.69	225.15	3.8
21	CML442/CZL2	2.513	75	2	182.5	87.5	0.476	0.830	10.4	29.05	183.70	5.3
22	CML442/CML312SR	1.303	72.5	3	167.5	77.5	0.438	0.923	10.4	29.83	184.55	6.3
23	CML442/CZL068	2.639	73	3	167.5	75	0.452	0.533	10.9	33.68	210.65	4.8
24	CML442/CZL04006	2.547	75	2	165	80	0.462	0.712	10.99	30.23	200.38	5.0
25	CML444/CML502	2.736	75	5.5	205	107.5	0.524	0.923	11	31.63	181.00	5.0
26	CML444/CZL1	3.013	80	1.5	202.5	105	0.550	1.071	11.6	29.95	243.80	4.3
27	CML444/CZL2	2.643	77	2.5	172.5	82.5	0.528	0.875	10.7	23.98	236.20	4.3
28	CML444/CML312SR	4.973	75.5	3	165	87.5	0.515	1.131	10.7	30.62	236.15	4.3
29	CML444/CZL068	5.454	76	1	192.5	102.5	0.526	0.938	10.3	35.15	273.45	4.5
30	CML444/CZL04006	3.045	78	2.5	187.5	95	0.526	0.781	10.7	28.26	203.20	4.5
31	CML502/CZL1	2.283	81	0	175	80	0.444	0.967	10.6	21.03	185.40	5.0
32	CML502/CZL2	2.276	76.5	2.5	177.5	82.5	0.457	1.071	10.3	26.90	175.00	5.8
33	CML502/CML312SR	1.005	73	2	175	85	0.500	1.000	10.4	45.40	204.80	5.3
34	CML502/CZL068	2.759	73	5.5	167.5	70	0.438	1.094	11.1	33.63	218.50	4.3
35	CML502/CZL04006	1.510	74.5	3	167.5	80	0.485	0.970	10.2	25.38	152.90	5.0
36	CZL1/CZL2	2.085	76.5	3	185	85	0.500	0.817	10.7	25.47	219.20	4.5
37	CZL1/CML312SR	2.555	76	1	167.5	85	0.545	0.849	10.8	26.80	237.00	4.5
38	CZL1/CZL068	3.931	78	2	170	72.5	0.412	1.010	10.4	34.57	291.20	3.5

Entry	Pedigree	GY	AD	ASI	PH	EH	EPO	EPP	MOI	CHL	KW	SEN
39	CZL1/CZL04006	1.679	80.5	1	165	77.5	0.469	0.683	11.1	24.07	222.95	4.0
40	CZL2/CML312SR	0.291	80.5	20.5	97.5	50	0.471	0.481	10.6	17.69	171.05	6.0
41	CZL2/CZL068	3.129	72	3	157.5	75	0.533	0.938	10.4	35.20	259.60	3.8
42	CZL2/CZL04006	2.427	71.5	4.5	175	90	0.529	0.806	10.5	27.64	218.45	5.0
43	CML312SR/CZL068	2.541	71.5	3.5	167.5	92.5	0.548	1.004	10.6	32.24	267.85	4.5
44	CML312SR/CZL04006	4.621	73	2.5	165	82.5	0.471	0.936	10.6	38.71	275.25	3.8
45	CZL068/CZL04006	4.381	72	2	167.5	75	0.485	0.967	10.2	37.18	264.70	3.8
Mean		2.56	75.3	2.9	177	83.8	0.47	0.88	11.1	29.6	204.8	4.7
LSD		1.86	3.5	4.7	22.9	18.1	0.08	0.34	1.2	8.5	6.54	0.9
Mse		0.88	3.0	5.7	192.4	98.6	0.001	0.03	0.4	21.9	10.47	0.3
p		0.001	0.001	0.001	0.001	0.0001	0.007	0.0001	0.627	0.000	0.000	0.000

APPENDIX E: Grain yield and high parent heterosis for the single cross hybrids under optimal conditions.

Entry	Pedigree	Hybrid GY (t/ha)	High Parent GY	Heterosis
1	CML312/CML395	8.882	3.38	338.64
2	CML312/CML442	11.277	3.09	556.64
3	CML312/CML444	14.489	4.03	883.38
4	CML312/CML502	9.279	1.73	591.29
5	CML312/CZL1	11.713	2.26	604.69
6	CML312/CZL2	10.665	1.29	126.63
7	CML312/CML312SR	9.390	3.19	266.67
8	CML312/CZL068	11.660	3.47	236.89
9	CML312/CZL04006	8.582	2.83	348.84
10	CML395/CML442	11.074	2.59	236.70
11	CML395/CML444	11.226	3.19	314.63
12	CML395/CML502	9.262	2.02	162.22
13	CML395CZL1	10.507	1.95	153.75
14	CML395CZL2	9.400	2.54	229.22
15	CML395CML312SR	12.159	4.51	418.70
16	CML395/CZL068	11.646	1.35	31.10
17	CML395/CZL04006	12.184	3.60	367.53
18	CML442/CML444	11.998	2.51	434.09
19	CML442/CML502	9.109	0.82	74.49
20	CML442/CZL1	8.830	3.09	556.40
21	CML442/CZL2	10.790	2.51	340.84
22	CML442/CML312SR	10.018	1.30	49.79
23	CML442/CZL068	10.517	2.64	156.24
24	CML442/CZL04006	12.450	2.55	304.33
25	CML444/CML502	12.266	2.74	567.36
26	CML444/CZL1	11.922	3.01	634.89
27	CML444/CZL2	12.488	2.64	363.72
28	CML444/CML312SR	11.311	3.48	299.58
29	CML444/CZL068	13.355	4.29	316.40
30	CML444/CZL04006	8.811	3.05	383.38
31	CML502/CZL1	11.915	2.28	613.45
32	CML502/CZL2	9.359	2.28	299.34
33	CML502/CML312SR	7.859	1.00	15.47
34	CML502/CZL068	10.887	2.76	167.82
35	CML502/CZL04006	9.661	1.51	139.70
36	CZL1/CZL2	9.236	2.08	265.71
37	CZL1/CML312SR	9.870	2.55	193.67
38	CZL1/CZL068	11.400	3.93	281.63
39	CZL1/CZL04006	9.019	1.68	166.52
40	CZL2/CML312SR	1.025	0.29	-66.60
41	CZL2/CZL068	11.827	3.13	203.80
42	CZL2/CZL04006	8.797	2.43	285.24
43	CML312SR/CZL068	12.296	2.54	146.72
44	CML312SR/CZL04006	10.218	3.72	327.28
45	CZL068/CZL04006	10.626	3.38	228.30
Mean		8.36	2.64	256.33
LSD		0.62	1.52	11.58
MSe		0.46	0.69	10.47

APPENDIX F: Grain yield and high parent heterosis for the hybrids under low N conditions.

Entry	Pedigree	Hybrid GY (t/ha)	High Parent GY	Heterosis
1	CML312/CML395	3.378	0.77	404.78664
2	CML312/CML442	3.086	0.47	681.86505
3	CML312/CML444	4.032	0.41	1007.2427
4	CML312/CML502	1.728	0.25	686.52038
5	CML312/CZL1	2.255	0.32	915.625
6	CML312/CZL2	1.292	0.57	-16.350877
7	CML312/CML312SR	3.190	0.87	266.66667
8	CML312/CZL068	3.470	1.03	236.8932
9	CML312/CZL04006	2.828	0.63	479.26531
10	CML395/CML442	2.593	0.77	336.15337
11	CML395/CML444	3.193	0.77	490.90167
12	CML395/CML502	2.019	0.77	172.31911
13	CML395CZL1	1.954	0.77	238.35993
14	CML395CZL2	2.535	0.77	228.57143
15	CML395CML312SR	4.513	0.87	434.94253
16	CML395/CZL068	1.350	1.03	51.929727
17	CML395/CZL04006	3.600	0.77	453.2517
18	CML442/CML444	2.510	0.47	788.65248
19	CML442/CML502	0.820	0.47	117.38602
20	CML442/CZL1	3.085	0.47	620.99696
21	CML442/CZL2	2.513	0.57	603.62573
22	CML442/CML312SR	1.303	0.87	117.74713
23	CML442/CZL068	2.639	1.03	161.98613
24	CML442/CZL04006	2.547	0.63	309.52381
25	CML444/CML502	2.736	0.41	507.80488
26	CML444/CZL1	3.013	0.41	959.56794
27	CML444/CZL2	2.643	0.57	428.19048
28	CML444/CML312SR	3.476	0.87	533.45813
29	CML444/CZL068	4.289	1.03	250.00832
30	CML444/CZL04006	3.045	0.63	448.08466
31	CML502/CZL1	2.283	0.32	650.32143
32	CML502/CZL2	2.276	0.57	325.64411
33	CML502/CML312SR	1.005	0.87	-29.37931
34	CML502/CZL068	2.759	1.03	189.32039
35	CML502/CZL04006	1.510	0.63	206.7997
36	CZL1/CZL2	2.085	0.57	395.36508
37	CZL1/CML312SR	2.555	0.87	339.40887
38	CZL1/CZL068	3.931	1.03	266.1877
39	CZL1/CZL04006	1.679	0.63	287.04762
40	CZL2/CML312SR	0.291	0.87	-60.853859
41	CZL2/CZL068	3.129	1.03	358.97735
42	CZL2/CZL04006	2.427	0.63	251.77627
43	CML312SR/CZL068	2.541	1.03	280.24965
44	CML312SR/CZL04006	3.717	0.87	485.23481
45	CZL068/CZL04006	3.381	1.03	225.48867
Mean		2.56	0.62	569
LSD		1.86	0.94	16.32
MSe		0.88	0.63	8.59
p		0.001	0.0145	0.007