Combining Ability for Ear Prolificacy and Response of Prolific Maize (*Zea Mays L.*) Hybrids to Low Nitrogen Stress

By

Makhumbila Penny (14010900)

BSc Agriculture (Plant Production) University of Limpopo

A dissertation submitted in partial fulfilment of the requirements for the degree of Master of Science (MSc) in Agriculture (Plant Production)

School of Agriculture

University of Venda

South Africa

April 2018

Abstract

Smallholder farmers in Sub-Saharan Africa still obtain low grain yields in maize largely due to low soil fertility. The soils are inherently low in nitrogen (N) that is required for the proper development of the maize plant. Currently there are no commercial cultivars for low N tolerance locally. The combining ability approach can be used as a tool for breeding desirable cultivars. In order to improve grain yield in maize, it is important to consider ear prolificacy which is a major yield component. Therefore this study was designed to estimate combining ability in maize. Exotic germplasm from the International Maize and Wheat Improvement Center and the Institute of Tropical Agriculture as well as the local germplasm from the Agricultural Research Council was used in the study to generate crosses. One hundred and two crosses were evaluated together with a standard commercial check under low N and optimum N conditions. The specific objectives of the study were to determine general and specific combining ability for prolificacy among local and exotic inbred lines and evaluate the response of prolific hybrids to low N conditions. The hybrids were planted in the 2014/2015 summer season under irrigation in Potchefstroom, Cedara and Taung in field plots consisting of 0.75m x 0.25m spacing in a 0.1 alpha lattice design replicated twice. Data for agronomic attributes were recorded and subjected to analysis of variance using SAS version 9.1.3. Genetic correlations were analyzed using the Principal Components Analysis and factor analysis based on the correlation analysis and major traits. The results showed variation in agronomic performance among the inbred lines and their F1 hybrids. Inbred lines including TZEI63, T1162W, L15 and L17 showed positive GCA estimates for ear prolificacy at the different locations. Specific combining ability for prolific hybrids was positive at all locations and environments. The GCA:SCA ratio was close to unity; indicating that the number of ears per plant showed highly significant (P<0.01) correlation with grain yield. The hybrids showed ear prolificacy under the low N conditions. This trait can be used effectively in stress tolerance maize breeding programmes.

Keywords: general and specific combining abilities, inbred line, maize, prolificacy, grain yield.

Dedication

This dissertation is dedicated to both my parents, my father, the late Khaizen Mawewe Makhumbila and my mother Khataza Mthavini Makhumbila who did not only raise me but worked tirelessly for me to get the education they never had.

My siblings Jimmy, Renny and Ennie Makhumbila for the support but most of all this is dedicated to my older sister Elsie Makhumbila who has not only supported me but has been my back bone and inspiration to continue pursuing agricultural research.

Declaration

I, ______________________ hereby declare that the research for the degree of Master of Science in Agriculture submitted at the University of Venda is my own original work and has not been submitted previously to this or any other university. I further declare, that the sources cited or quoted herein have been duly acknowledged by means of complete list of references.

Signature………………………………… Date…………………………………………

(Candidate: Makhumbila, P.)

As the candidate's supervisors, we agree to the submission of this dissertation:

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Supervisor: Prof. E.T. Gwata

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Co-Supervisor: Dr. K. Mashingaidze

Acknowledgements

I would like to acknowledge my supervisors, Prof. E.T. Gwata and Dr. K. Mashingaidze for valuable contribution towards this study. Their continued support and guidance is highly appreciated. I also acknowledge the Agricultural Research Council - Grain Crops Institute, National Research Foundation and University of Venda for the financial support for this study. Carrying out this work would not have been possible without the help of my colleagues Sphiwokuhle, Lesedi, Jabulile and Willy, all the support given throughout the study is appreciated. Monica, thank you for always believing in me even when I didn't believe in myself.

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List of Abbreviations

ARC : Agricultural Research Council

ASI : Anthesis silking Interval

CIMMYT : International Maize and Wheat Improvement Center

ED : Ear diameter

EH : Ear height

EL : Ear length

FW : Field weight

GCA : General combining ability

GCA$_f$: General combining ability due to females

GCA$_m$: General combining ability due to males

GCI : Grain Crops Institute

GY : Grain yield

IITA : International Institute of Tropical Agriculture

L : Female inbred line

LAN : Limestone Ammonium Nitrate

M : Male inbred line

N : Nitrogen

NCII : North Carolina Design II

NEP : Number of ears per plant

NPK : Nitrogen, Phosphorus and Potassium

PCA : Principal components analysis

PHT : Plant height

SCA : Specific combining ability

SP : Shelling percentage

SSA : Sub-Saharan Africa

CHAPTER 1: GENERAL INTRODUCTION

1.1 Origin of maize

Maize (*Zea mays*) belongs to the family Poaceae and originated in Central America in Mexico. Archaeological records suggest that domestication of maize began at least 6000 years ago (Freitas *et al.,* 2003). Maize is cultivated widely in the tropics, sub-tropics and temperate regions of the world including Africa, America and Asia (Morris, 1998). Since its introduction in the 1500s, maize has become one of Africa's most dominant crops.

It is widely grown in Latin America and Africa where it has become an important cereal crop. In Africa, it is cultivated by both large-scale and smallholder farmers. It is regarded as both a cash and subsistence crop. In Sub-Saharan Africa (SSA), maize covers over 25 million hectares and the smallholder sector produces approximately 38 million metric tons for food (Smale *et al.*, 2011).

1.2 Uses of maize

Maize has numerous uses. It is used mainly for human consumption and animal feed. It is consumed differently in different continents, while its most popular products are flour and meal (Tasfaye *et al*., 2016). In some countries, maize is used as a biofuel. The use of biofuel derived from maize reduces carbon dioxide emissions and thus contributing to slowing down global warming effects (Crutzen *et al.,* 2008). Other industrial uses of maize include the production of starch, oil, beverages, glue and alcohol (Ranum *et al.,* 2014)

1.3 Nutritive attributes of maize

One of the reasons for dependence on maize in many countries of SSA is that there is nutritional security in the crop. Maize contains approximately over 70% of starch, 10% protein and 4% fat. It supplies ample amounts of energy to both the human and animal body. Since its domestication, it has been a popular source of nutrients (Ranum *et al.*, 2014; Tasfaye *et al*., 2016). In many developing countries, maize plays a crucial role in diets by providing a high content of carbohydrates, fats, proteins, crucial vitamins and minerals.

Maize provides vitamins A and B, it is however low in usable protein and its leucine inhibits the human body's absorption of niacin; which is a vitamin that causes protein deficiency when absent. The research institute for International Maize and Wheat Improvement Center (CIMMYT) developed maize germplasm with enhanced levels of lysine and tryptophan in the endosperm and a better amino acid balance (Wegary *et al.*, 2011).

1.4 Main production constraints

In many rural communities of the Sub-Saharan Africa, farmers use manure as a source of nutrients for their crops including maize. However, due to volatilization and leaching, high concentrations of major nutrients such as nitrogen (N) are lost (Smith *et al.*, 2014; 2015). Marungu *et al.*, 2011 highlighted the low usage of both chemical nitrogenous and organic fertilizers particularly in Africa. In South Africa, the annual maize consumption is about 9 million tonnes. The crop is produced mainly by large-scale commercial farmers (>90%) while the remainder is produced by the smallholder farmers. However, the smallholder sector is constrained by many factors including limited arable land and poor infertile soils. In addition, the use of mineral fertiliser is still limited in the smallholder sector. In a survey conducted in Limpopo Province (South Africa), 75% of smallholder farmers could not afford sufficient quantities of chemical fertiliser that are recommended for the crop (Odhiambo and Magandini, 2008).

Although there are usually fertiliser subsidies provided by the government for these smallholder farmers, the amount of fertiliser allocated per farmer is insufficient to sustain the crop throughout the growing season (Marenya *et al.*, 2012). However, the seed which is usually distributed to the smallholder sector is produced from varieties developed particularly for commercial farmers who have access to resources required for successful production of the maize crop (Fischer and Hajdu, 2014).

1.5 Productivity and nitrogen requirement

Generally, the productivity of the maize crop is measured by the amount of grain produced per unit area. Therefore, maize genotypes that have high grain yield per unit area are described as highly productive. The genotypes that produce more than one ear (or cob) per plant are termed prolific. These prolific maize genotypes have been reported in several studies (Harris *et al.*, 1976; Varga *et al.*, 2004). However, there is inadequate information pertaining to the performance of these prolific genotypes in poor (infertile) soils particularly in the smallholder sector in South Africa.

In particular, inorganic nitrogenous fertilisers are essential in the growth and development of maize. Nitrogen is a major nutrient for maize development as it is a constituent element of cell contents. In numerous studies, N has been appraised for its ability to delay senescence of the maize crop (Muchow, 1988; Uhart and Andrade, 1995b). The maize plant takes up N rapidly at the middle vegetative growth stage (V10) and the maximum N uptake occurs near the silking stage. During flowering, the plant requires sufficient amounts of N to produce kernels. However, due to reduced photosynthetic activity during this stage kernel setting is often poor particularly if the genotype is not tolerant to low N stress conditions (Uhart and Andrade 1995a; Zhang *et al.*, 2007). In most smallholder maize production systems, the soils are deficient in N and this limits yields (Sanchez, 2002; Smith *et al.*, 2014). Therefore, development of maize genotypes that can produce stable yields under low N conditions is essential. Identification of such genotypes will benefit smallholder farmers and enhance food security.

1.6 Problem statement

Smallholder farmers grow maize in soils that are inherently infertile and cannot afford inorganic fertilisers readily. This leads to low yields (<3.0 t/ha). Hybrid selection is usually conducted under high yielding conditions where heritability for prolificacy is high. In these instances, combining abilities of the parental lines of the hybrids under low N stress is usually ignored. Prolificacy of maize is associated with tolerance to drought and high plant populations. However, there is no information on the performance of prolific hybrids under low N stress conditions. There is also a lack of information on the combining abilities of South African inbred lines with exotic inbred lines. Sufficient understanding of parental combinations (combining ability) is essential for successful breeding of improved maize cultivars that are tolerant to low N stress and can produce stable yields even under low N stressed environments.

1.7 Rationale of the study

A maize breeding approach which utilizes genetic information generated from evaluating combining abilities of parental genotypes is useful for efficient cultivar development. This will enhance the identification of parental line combination that can produce stable yields under low N stress conditions. The utilization of such germplasm in cultivar improvement will benefit maize production in the smallholder sector of South Africa and end-users.

1.8 Research objectives

The broad objective of the study was to evaluate the performance of maize germplasm under infertile conditions. The specific objectives of the study were to determine the following:

(i) general combining ability (GCA) for prolificacy among selected local and exotic maize inbred lines

(ii) specific combining ability (SCA) for prolificacy between selected local and exotic maize inbred lines

(iii) response of prolific hybrids to both optimum and low N stress

1.9 Hypotheses

The study tested the following null hypotheses:

(i) there are no significant differences in GCA among local and exotic maize inbred lines.

(ii) there are no significant differences in SCA among local and exotic maize inbred lines.

(iii) maize ear prolificacy under stress is absent.

1.10 Dissertation outline

This dissertation consists of five chapters. Chapter one is the general introduction that introduces maize as a crop and its contribution to food security. It further introduces N as a maize crop production constrain and therefore drawing the objectives of the study and the hypotheses tested. The second chapter of the dissertation reviews literature associated with combining abilities and other aspects of maize that are crucial in stress tolerance breeding. Chapter three consists of materials and methods utilised in the study. Chapter four presents the results of the study. Chapter five discusses the research findings and the last chapter focusses on concluding remarks and some recommendations.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Maize (*Zea mays* L.) (2n = 20) is a monoecious annual plant. Its flowers are borne on the same plant. The male flower is called the tassel and the female part is called the ear. At the fifth growth stage of the plant, the ear produces silk and the tassel sheds pollen. The maize ear terminates one or more lateral branches, usually halfway up the stem. The ear is enclosed in bracts and as soon as silk appears on the upper part of the ear, it is receptive to pollen (Freeling and Walbot, 1994). Some varieties can produce more than one ear and this is called ear prolificacy (Varga *et al.*, 2004).

Maize leaves are arranged spirally and occur alternately on the stem. The leaf is a typical grass leaf consisting of a sheath, legules, auricles and a blade. The entire leaf is supported by a long prominent mid-rib. The roots are profusely branched and the root system consists of adventitious roots and prop roots (Ning *et al.*, 2014).

Maize is a warm weather crop. It is cultivated in areas where the mean daily temperature is $\geq 19^0$C. The crop requires adequate supply of moisture for development (Itabari *et al.*, 1993). However, the development varies between hybrids, seasons, time of planting, location, water availability and soil fertility status.

2.2 Physiological influence of low nitrogen on the maize plant

Nitrogen (N) is a critical plant nutrient and can be considered as a major yield determinant factor in the production of maize. For optimum maize production, it is essential for N to be in sufficient quantities (Biswas and Mukherjee, 1993; Kogbe, 2003). In studies that were carried out under low N conditions, low quantities of N had a negative impact on the maize plant particularly in the reduction of the leaf area which in turn decreases the photosynthetic rate (Aderiran and Banyoko, 1995; Shanti *et al.,* 1997). Uhart and Andrade (1995a, b) reported that deficiency in N delayed both vegetative and reproductive development of the plant, reduced leaf emergence rate and diminished leaf expansion rate. Reduced leaf area index, light interception, biomass production, kernel number and grain yield were also attributed to N deficiency.

Metabolic compounds essential for proper cell function in the maize plant such as amino acids, proteins, enzymes, co-enzymes and some of the non-proteinaceous compounds require N (Biswas and Mukherjee, 1993). The biochemical properties of maize leaves are greatly affected under low N conditions. The chlorophyll content, nitrate reductase and glutamate synthase which are enzymes closely related to leaf senescence are also affected in this phenomenon (Bertin and Gallais, 2000). In poor N conditions, the maize leaf blade changes to yellow due to chlorophyll degradation (Buchanan-Wollaston *et al.*, 2003). Low N can also result in poor ear and kernel development or even abortion thus leading to poor yield (Muchow and Sinclair, 1994). The reduction in leaf area, chlorophyll content and vegetative biomass affect the overall yield of the maize crop (Uhart and Andrade, 1995b; Papanov and Engels, 2003; Monneveux *et al.*, 2005; Echarte et al., 2008; Ciampitti and Vyn, 2011). The critical stage of N requirement in maize starts at the middle vegetative growth stage when the 4th – 12th leaves unfold. Hanway, (1963) and Settimi *et al.,* (1998) observed that when the crop is at flowering stage, the usage of N increases. The status of N affects numerous plant processes and during the flowering stage and vegetative stage, N is essential for good development of the plant (Ciampitti and Vyn, 2011). Despite these numerous symptoms caused by N deficiencies, there are maize genotypes that can withstand these conditions and such genotypes are of interest to maize breeders.

2.3 Maize yield components

2.3.1 Ears per plant

The number of ears per plant of the maize crop is of importance to yield determination of a genotype. Ear prolificacy is the ability of a maize genotype to produce more than one ear per plant. This trait has been of great interest to breeders. Ear prolificacy was highly correlated with yield in maize (Goodman, 1965). Prolific hybrids tend to resist barrenness at high populations and also show good stability over a wide range of environments (Collins, W.K. *et al.*, 1965; Varga *et al.*, 2004). Moll, *et al.,* (1986) developed single cross hybrids that produced more than two cobs per plant under favorable conditions. In their study, it was observed that under stress conditions, hybrids that were prolific produced one or two cobs that reached maturity. Hence they tend to resist barrenness unlike nonprolific hybrids. Prolific hybrids extract more water from the soil when under moisture stress (Barnes and Woolley, 1969). Under low N stress conditions, prolific hybrids take up N more efficiently and rapidly from the soil than nonprolific hybrids. Hence they can minimize barrenness and produce kernels on both the first and second ear (Collins *et al.*, 1965; Moll *et al.*, 1982; Varga *et al.*, 2004).

2.3.2 Ear length and diameter

The maize ear length and diameter are directly associated with the grain yield of a genotype. An increase in the ear length and diameter under nitrogenous conditions increased the yield (Pursushttam and Puri, 2001), suggesting that this trait requires attention in maize improvement programmes aimed at increasing the grain yield. Longer ears with more diameter improve kernel setting and kernel weight, therefore improving yields. The absence of N during the physiological growth of the maize plant results in poorly developed ears. Thus compromising the ear length and diameter, resulting in yield reduction (Shaharoona *et al.,* 2006).

2.4 Maize as a source of oil, starch and protein

2.4.1 Oil content

The development of maize genotypes with high oil content dates back to the 1960's. Maize oil is high in energy and often livestock farmers prefer this quality as the oil extracted from maize gives energy over an extended period, thus saving feed costs. In the starch industry, oil contributes to the quality of the product sold to consumers. Over the years' extraction methods have also been advancing and the industry has thus been expanding for maize oil products (Matsakidou *et al.,* 2015, Vȧzquez-Carrillo *et al.*, 2014; 2015). The nutritional properties of processed maize oil has been appraised as it is easily digestible and contain oily compounds, such as vitamins that are essential for healthy skin. For cholesterol control, dieticians often recommend the use of maize oil in households. The oil can be used in the production of many industrial products used daily in households; cooking oil, mayonnaise, salad dressing, soap and other pharmaceutical products (Jugenheimer, 1961). The quality of both traditional and commercial maize production products depends on certain endosperm properties and the properties include both oil and protein. An increased N application rate has been found to increase the oil percentage of maize. Under low N stress conditions, the oil content is reduced enormously when compared to high N conditions. Oil content is thus significantly affected by N availability and genotype (Oiken *et al.,* 1998).

2.4.2 Starch content

The starch content of maize is important in the food production industry as it is often used as an end product in the milling industry. In Sub-Saharan Africa (SSA), maize milling is popular both at commercial and small-scale. Although uses and preparations of the milled maize is different, maize starch is a constituent of numerous food products that contribute nutrients (Shenghua *et al.*, 2014; Abia *et al.,* 2017). Starch aids in the formation of short-chain fatty acids that are essential for energy production for both humans and animals (Jin *et al.,* 2017). The biochemical properties of maize starch are of importance in biofuel production. Its properties add value to the end product, therefore enhancing the economy of the biofuel industry (Yigiu *et al.,* 2013).

The unavailability of ample quantities of N for the maize plant affects the growth stages of the plant. Therefore, resulting in poor kernel formation and reduced kernel sizes. This reduces the starch quality and quantity of the maize kernel (Uhart and Andrade, 1995a, b).

2.4.3 Protein content

Maize plays a vital role in the nutrition of both humans and animals in developing countries worldwide. Breeders worldwide did not pay much attention to breeding for protein until the 1960's where a major discovery was made. During this time, the presence of mutants such as opaque-2 was made. The mutant produces enhanced levels of lysine and tryptophan, which are the two amino acids deficient in maize (Prasanna, 2001). Maize is the most dominant crop in in most parts of Africa, particulaly in the SSA. It is widely grown by both smallholder and commercial farmers. Most smallholder farmers in rural areas rely on the crop for nutritional gains. However, nutritionally, the crop is deficient in two crucial amino acids, lysine and tryptophan, which contribute to the quality of the protein produced in maize (Bhatia and Rabson, 1987). High protein genotypes have the ability to supply more amino acids to their kernels and have an enhanced expression of enzymes that contribute greatly to high quality protein accumulation (Doehlert and Lambert, 1991). Protein is also affected by the quantity of N supplied to the plant during its growth stages. Very little work has been reported particularly in South Africa, pertaining to the impact of N stress on protein percentage composition of hybrids.

2.5 Sources of nitrogen in smallholder sector

2.5.1 Cover cropping, intercropping and rotation with legumes

Legumes are considered a key component of integrated soil fertilization management strategies (ISFM) for smallholder farmers in SSA because they can fix atmospheric N (Vanlauwe *et al.,* 2010). The use of N fixing legumes as a cover crop has been promoted widely as cover crops in SSA to improve soil fertility and crop yields. Maize yields have also been improved in the smallholder sector of many parts of Africa through the use of legumes as cover crops. The use of legumes as cover crops has its advantages as it reduces fertilizer input costs (Buresh and Tian, 1998; Kiptot *et al.*, 2007; Tully *et al.,* 2015). Herbaceous legumes are the commonly grown as they fix atmospheric N_2, thus complementing mineral fertilizer use and also increasing carbon (C) stocks (Sanchez, 2002).

Maize is often intercropped with legumes in the smallholder sector in many parts of Africa. These legumes conserve moisture and provide N for the maize crop through N fixation (Andersen *et al.*, 2005). Intercropping of cereal crops like maize has now become of major interest to scientist in order to try increase yields in the smallholder sector (Jensen, 1996; Neugschwandtner and Kaul, 2015). The yields of crops grown in an intercropped system increases due to increased nutrient uptake especially when legumes are intercropped into the field. Sole crop stands give poor yields when compared to legume intercropped fields (Coll *et al.,* 2012; Regehr *et al.,* 2015). Rotation of maize with legumes has been appraised for increasing maize yields significantly as compared to having a fallow period before growing the crop. A fallow period has its disadvantages as nutrients can be lost to leaching. Having a rotation with legumes can be a good way of preserving these nutrients, particularly N (Carsky *et al.,* 1999; Yasuf *et al.*, 2009; Ojiem *et al*., 2014). The subsequent increase of yields in a crop rotation system with legumes can be attributed to biological nitrogen fixation and other rotation beneficial effects. Some of the other beneficial effects in a legume rotation system include enhancement of soil microbial activities and further enhancing the chemical properties of soil (Baldock *et al.*, 1981; Yasuf *et al.*, 2009).

2.5.2 Organic fertilizers

The source of fertilizer for most smallholder farmers is kraal manure, compost and leaf litter. For many smallholder farmers, organic fertilizer is an effective substitute for inorganic nitrogenous fertilizers (Svotwa *et al.*, 2009). The use of organic means of fertilization has numerous benefits, particularly its ability to improve the soil structure and water retention (Tisdal and Oades, 1982). Although organic matter is an important source of nutrients for plant production, it is often too bulky and expensive to transport (Cambardella and Elliot, 1992). Nitrogen availability in farming systems that utilize organic fertilizers as the main source of nutrients is often poor as N availability is dependent on many factors including the biodegradability of the organic material used in the field (Gregorich *et al.,* 2003).

2.6 Breeding for tolerance to low nitrogen stress

Hybrids that can use N efficiently have been developed through selection for the stay green trait (Yan *et al.*, 2014). Nitrogen tolerant maize cultivars tend to have a decreased leaf senescence rate. During flowering, N tolerant genotypes have a decreased ear abortion and also have an increased assimilate supply essential for grain filling (Lafitte and Edmeades, 1994; Bänziger *et al.*, 2002).

Maize cultivars tolerant to low N stress have been evaluated at different nitrogen rates and it was found that, an increased N application rate increased the N uptake by the plant (Azeez *et al.* 2006). Low N stress results in yield levels of about 25 – 35% when compared to the yields obtained under well-fertilised conditions (Weber *et al.*, 2012). Breeders often select best performing hybrids under optimum conditions not taking the smallholder farmers' constraints into account. However, selection of best performers under low N stress conditions can be ideal as both smallholder farmers and commercial farmers can have improved yields.

The Institute of Tropical Agriculture and the International Maize and Wheat Improvement Centre have developed elite germplasm tolerant to low N stress conditions available for breeders to use (Weber *et al.*, 2012). However, the combining abilities of these exotic lines with South African lines is not known.

2.7 Mating designs: North Carolina Design II

Successful plant breeding requires selection of suitable mating design. According to Khan *et al.*, (2009) a mating design is a method of producing progenies that can be evaluated over a range of environments. Commonly used designs include the Diallel (I, II, III, IV) (Griffing, 1956), Line X Tester (Kempthorne, 1957) and the North Carolina (NC) (I, II, III) (Comstock and Robinson, 1952).

The NCII uses blocking that allows all mating combinations involving a single group of males to a single group of females to be kept intact as a unit. Each member of a group of parents used as males is mated to each member of the group of parents used as females (Nduwumuremyi *et al.*, 2013). This is a factorial mating scheme and the conceptual and practical method of mating was defined by Acquaah, 2012 (Fig.1). The NC II design provides estimates of specific and general combining ability. It also provides heritability of traits that can be of importance to the breeder. Such estimates can be used to identify the best female and male inbred lines with better performing hybrid combinations.

2.8 General and specific combining ability

The theory of general combining ability (GCA) and specific combining ability (SCA) established by Sprague and Tatum (1942) have been used broadly in breeding of several crop species including maize. The average performance of a strain or genotype in a series of hybrid combinations is termed GCA. It helps in the selection of good inbred parents for hybridization and is related to narrow sense heritability. The SCA is the performance of a parent in a specific cross. The SCA aids in the identification of superior cross combinations and has a relationship with heterosis (Falconer and Mackay, 1996). GCA is largely due to the additive effect of genes while SCA is associated with dominance or epistatis effect of genes.

Combining ability is a pre-requisite for development of good economically reliable hybrids in maize. It is useful in assessing the potential of inbred lines and identifying the gene action involved in the inheritance of various quantitative characters. Information on combining ability among maize germplasm is essential in maximising the effectiveness of hybrid development. The GCA and SCA variances provide estimation for additive gene actions (Ai-zni, *et al.*, 2012). Therefore, it is necessary to evaluate the combining abilities of inbred lines that are used as parents in breeding programmes in order to develop high yielding cultivars that are tolerant to abiotic and biotic stress (Le Gouis *et al.,* 2002; Machikowa *et al.*, 2011; Chigeza *et al.*, 2014).

The combining ability approach has been used successfully in maize (Colbert *et al.*, 1987; Aliu *et al.*, 2008; Izhar and Charkraborty, 2013; Akinwale *et al.* 2014; Bertoia and Aulicino, 2014; Ketthaisong *et al.*, 2014), sorghum (Makanda *et al.*, 2010) and sunflower (Machikowa *et al.*, 2011) among other crops where SCA and GCA have been found to be significant for desired traits. SCA and GCA estimates are statistically robust and can aid the breeder improve genotypes.

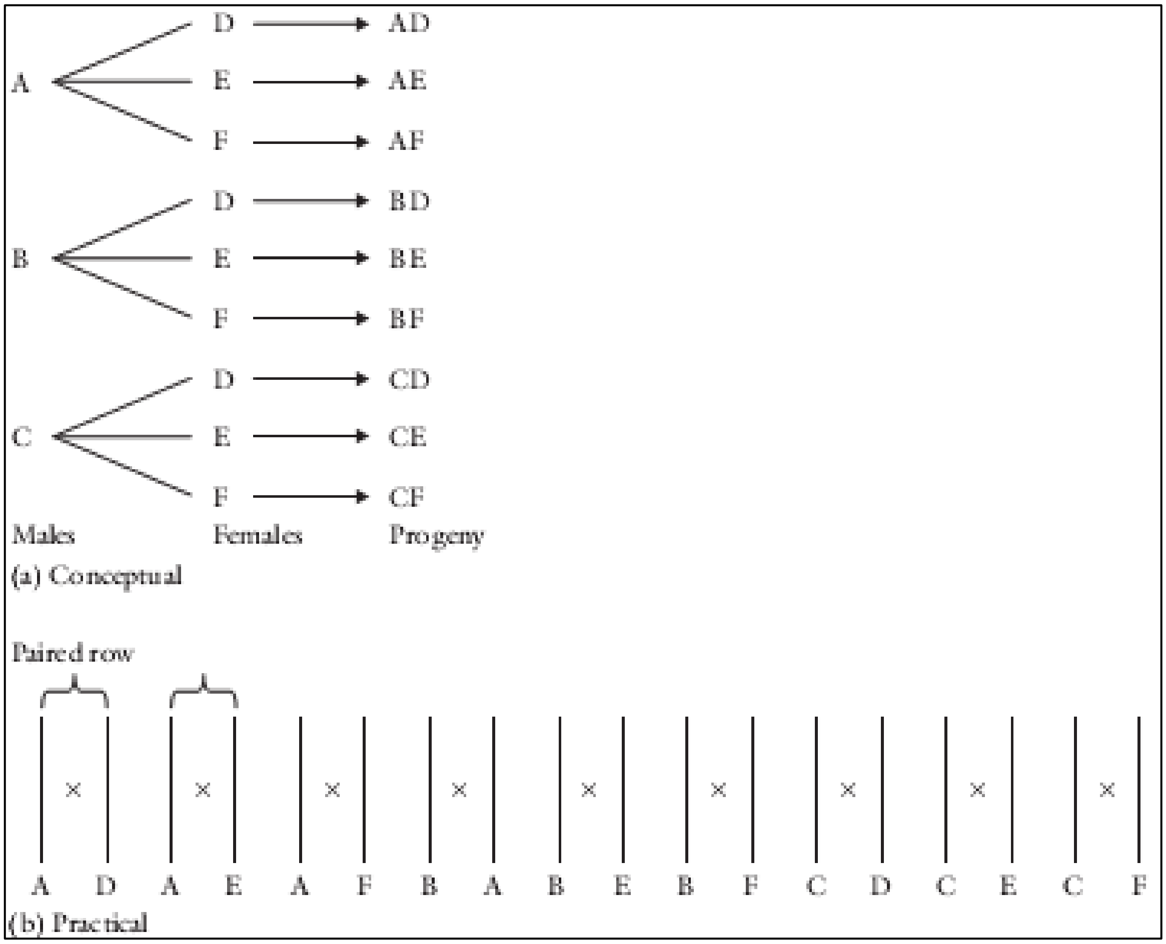

Fig. 2.1 NC II design (factorial design with paired rows). Acquaah, 2012.

CHAPTER 3: MATERIALS AND METHODS

3.1 Hybridization and experimental sites

The initial stage of this research work used Makhathini Research station (altitude of 77m, temperatures of 15 – 29^{0}C and rainfall of about 336 mm) for developing the maize hybrids that were used for the study. The location of Makhathini was used because it is frost free hence it enabled the hybrid development during the winter (may/June) season 2014.

The hybridization process included covering the ear of each female parent prior to silk emergence. The ear shoot was bagged/covered using transparent plastic bags (Fig. 3.1) in order to prevent contamination by undesirable pollen. The Tassel on each plant was also bagged in order to collect pollen for subsequent manual transfer onto the emerged silk of the selected female parent. The pollinated plant was then covered with a brown paper bag until physiological maturity to prevent undesirable pollination (Richey, 1927; Bannert. 2007).

Fig. 3.1 Procedure of hybrid development showing (a) ear shoot bagging and (b) emerged silk ready for pollen transfer.

The second phase of the study evaluated the hybrids at three testing locations (Table 3.1). Each of the locations was in high altitude (>1000.00 above sea level) and received >300.0 mm of rain annually on average.

Table 3.1 Testing locations that were used for evaluating maize hybrids.

Name of location (province)	**Mean**		**Rainfall (mm)**	**Environmental type**
	Altitude (m)	**Temperature ^{0}C**		
Potchefstroom (North West)	1349.0	15.0 - 30.0	320.0	Low N and optimum
Cedara (KwaZulu-Natal)	1033.0	12.0 - 25.0	355.0	Optimum
Taung (North West)	1116.0	16.0 - 31.0	334.0	Low N and optimum

3.2 Genetic material

Elite inbred lines tolerant to low N stress and found prolific under optimum conditions were obtained from the International Maize and Wheat Improvement Center (CIMMYT), the International Institute of Tropical Agriculture (IITA) and the Agricultural Research Council-Grain Crops Institute (ARC-GCI). Hybrid combinations were made among inbred lines (Table 3.2 and Table 3.3). Altogether, 102 hybrids were developed using NCII (see section 3.3 below). In addition, three local checks from seed companies were included in the field evaluation component of the study.

Table 3.2 List of 23 male inbred lines used to generate F1 hybrids using NCII mating design.

Name	**Source**	**Trait**
I-20	ARC	Prolific
I-34	ARC	Prolific
I-35	ARC	Prolific
CB232	ARC	Prolific
CB255	ARC	Prolific
CB299	ARC	Prolific
RO544W	ARC	Non-prolific
RO549W	ARC	Non-prolific
T1162W	ARC	Non-prolific
J80W	ARC	Non-prolific
M37W	ARC	Non-prolific
RO452W	ARC	Non-prolific
CML538	CIMMYT	Drought + low N tolerant
CML545	CIMMYT	Drought + low N tolerant
CML489	CIMMYT	Drought + low N tolerant
CML521	CIMMYT	Drought + low N tolerant
CML546-B	CIMMYT	Drought + low N tolerant
TZEI 56-B	IITA	Drought + low N tolerant
TZEI 5-B	IITA	Drought + low N tolerant
TZEI 63-B	IITA	Drought + low N tolerant
TZEI 11-B	IITA	Drought + low N tolerant
TZEI 13-B	IITA	Drought + low N tolerant
TZEI 14-B	IITA	Drought + low N tolerant

Table 3.3 List of 24 female inbred lines used to generate F1 hybrids in NCII mating design.

Name	Code	Source	Trait
I-39	L21	ARC	Prolific
T1162W	L22	ARC	Prolific
I-42	L1	ARC	Prolific
CB323	L2	ARC	Prolific
CB388	L3	ARC	Prolific
CB400	L4	ARC	Prolific
M162W	L5	ARC	Non-prolific
R2565Y	L6	ARC	Non-prolific
U2540W	L7	ARC	Non-prolific
FO215W	L8	ARC	Non-prolific
RO549	L9	ARC	Non-prolific
VO500Y	L10	ARC	Non-prolific
CML548	L13	CIMMYT	Drought + low N tolerant
CZL03021	L11	CIMMYT	Drought + low N tolerant
CML523	L12	CIMMYT	Drought + low N tolerant
CML547	L16	CIMMYT	Drought + low N tolerant
CZL068	L14	CIMMYT	Drought + low N tolerant
TL117077	L15	CIMMYT	Drought + low N tolerant
TZEI 7	L17	IITA	Drought + low N tolerant
TZEI 83	L24	IITA	Drought + low N tolerant
TZEI 83	L23	IITA	Drought + low N tolerant
TZEI 161	L18	IITA	Drought + low N tolerant
TZEI 173	L19	IITA	Drought + low N tolerant
TZEI 175	L20	IITA	Drought + low N tolerant

The field trials in the different locations under the following environments:

(i) Potchefstroom low N

(ii) Taung low N

(iii) Potchefstroom optimum

(iv) Cedara optimum

3.3 Experimental design

The F1 hybrids were developed using the NCII mating design as illustrated below (Table 3.4). Each of the male inbred line was crossed to six randomly selected female inbred lines.

Table 3.4 Schemes of parental combinations that were used for creating the F1 hybrids.

Male Parent	**Female Parent**					
	L1	L2	L3	L4	L5	L6
M1	L1M1	L2M1	L3M1	L4M1	L5M1	L6M1
M2	L1M2	L2M2	L3M2	L4M2	L5M2	L6M2
M3	L1M3	L2M3	L3M3	L4M3	L5M3	L6M3
M4	L1M4	L2M4	L3M4	L4M4	L5M4	L6M4
M5	L1M5	L2M5	L3M5	L4M5	L5M5	L6M5
M6	L1M6	L2M6	L3M6	L4M6	L5M6	L6M6
M7	L1M7	L2M7	L3M7	L4M7	L5M7	L6M7
M8	L1M8	L2M8	L3M8	L4M8	L5M8	L6M8
M9	L1M9	L2M9	L3M9	L4M9	L5M9	L6M9
M10	L1M10	L2M10	L3M10	L4M10	L5M10	L6M10
⋮	⋮	⋮	⋮	⋮	⋮	⋮
M23	L1M23	L2M23	L3M23	L4M23	L5M23	L6M23

L = female inbred line *M = male inbred line*

In the field evaluation, the hybrids (102) and a standard commercial check were laid out following the (0,1) α lattice design replicated twice with 42 blocks. The hybrids together with the local checks were evaluated across locations under low N and optimum conditions.

3.4 Trial establishment and management

Inbred lines were planted in a 4.0m row, 4 row field for plots with 0.25m x 1.0m inter- and intra-row spacing, respectively at Makhathini Research Station. In order to synchronize silking and flowering, only two rows were planted first in each plot and the remainder were planted two weeks later. Two seeds were planted at each station and subsequently thinned (at knee height stage) to one plant per station. Basal fertilizer of NPK; 2:3:4 was applied before planting (40.0 kg/ha) and Limestone Ammonium Nitrate was also applied as top dressing immediately after thinning. The off-season nursery for hybrid development was irrigated as necessary throughout the winter season. Standard weed control methods for maize production were followed throughout the season.

3.4.1 Optimum testing locations

Hybrids evaluated under optimum conditions were planted in 4.0m rows with each plot consisting of two rows. The plots had 0.25m x 1.0m inter- and intra-row spacing, respectively with two seeds planted in each station. From the two plant stationed together, thinning was carried out to even out the competition between the plants. Basal fertilizer of NPK (2:3:4) was applied before planting and later followed by application of LAN (du Plessis, 2003). Irrigation was carried out throughout the development of the crop. Weed control was achieved through the use of herbicides and mechanical weeding. At harvest maturity, plots were harvested by hand separately per replication.

3.4.2 Low nitrogen testing locations

Hybrids evaluated were planted in 4.0m rows with each plot consisting of two rows. The plots were 0.25m x 1.0m spacing and two seeds planted in each station where a single plant was thinned out at a later stage when plants had geminated. In order to obtain at least 20.0% of the normal yield 10.0 kg/ha of LAN was applied at planting only. Potassium Chloride and double Super Phosphate were applied at 50.0 kg/ha at planting. All plant matter in the field during both weeding and thinning were removed to avoid adding any form of N deposit to the soil through organic matter. Straws from the previous season were also gathered and burnt. In the low N blocks utilized for planting, there were no previous recent rotations or plantings of leguminous crops to intensify the N stress level.

3.5 Measurements

The following parameters were measured:

(i) Plant height (PHT) – this was measured from the ground level to the point of the first insertion of the tassel

(ii) ear height (EH) – measured from the ground to the insertion of the first active ear

(iii) duration to flowering – this was measured as the number of days from planting to when 50.0% of the plants per plot have extruded anthers and when 50.0% are showing silk. From this the anthesis silking interval (ASI) was calculated

(iv) ear length (EL) – the length of cobs without husk covers was measured in centimeters (cm)

(v) ear diameter (ED) – direct diameter measurements were made with the use of a Vernier caliper (cm). The diameter was measured on sampled cobs without husk covers

(vi) number of plants per plot

(vii) number of ears per plot – a total count of all cobs belonging to a single plot. A measure of this variable together with the number of plants per plot were used to determine the average number of ears per plant (No.EPP)

(viii) grain moisture (MOI %) – measured after shelling of respective plots using a moisture meter at harvest

(ix) field weight (FW) – the weight of all cobs belonging to a single plot was measured in kilograms (kg)

(x) grain weight – measured after shelling (kg) at harvest

(xi) grain yield (GY) – the grain yield was converted to tons/ha using the following formula:

$$\left(\frac{GWx10}{Plot\ Area}\right)x\left(\frac{100-MOI}{100-12.5}\right)$$

3.6 Statistical analyses

Analysis for combining abilities was conducted using SAS (2005) software with Restricted Maximum Likelihood covariance estimates. The model used for SCA and GCA estimates was NCII design:

$$Y_{ijk} = \mu + M_i + F_j + MF_{ij} + R_k + \varepsilon_{ijk}$$

Where

Y_{ijk} = observed trait value

μ = mean effect

M_i = effect of the i^{th} male

F_j = effect of the j^{th} female

MF_{ij} = effect of interaction between the i^{th} and j^{th} female

R_k = effect of the k^{th} replication

ε_{ijk} = experimental error

Genetic correlations were analyzed using the Principal Components Analysis (PCA) and factor analysis based on the correlation analysis and major traits.

CHAPTER 4: RESULTS

4.1 Production and performance of prolific hybrids

The nursery that was established at Makhathini Research Station for the creation of hybrids was successful (Fig. 4.1). The subsequent hybridization through manual pollination, produced 102 hybrids. In the field trial trials in both optimum and low N conditions, the hybrids showed ear prolificacy (Fig 4.2). In some cases, individual hybrid plants produced more than two extra ears but such ears were inferior in comparison with the main ear on the plant (Fig. 4.3). In general, the multiple ears varied in both size and kernel load. In addition, some hybrids showed poor seed set and diminished ear sizes (Fig. 4.4). Nonetheless, there was adequate seed (from all the hybrids) to conduct field trials and determine the combining abilities.

Fig. 4.1 The nursery of maize germplasm at the hybridization stage at Makhatini Research Station, 2014.

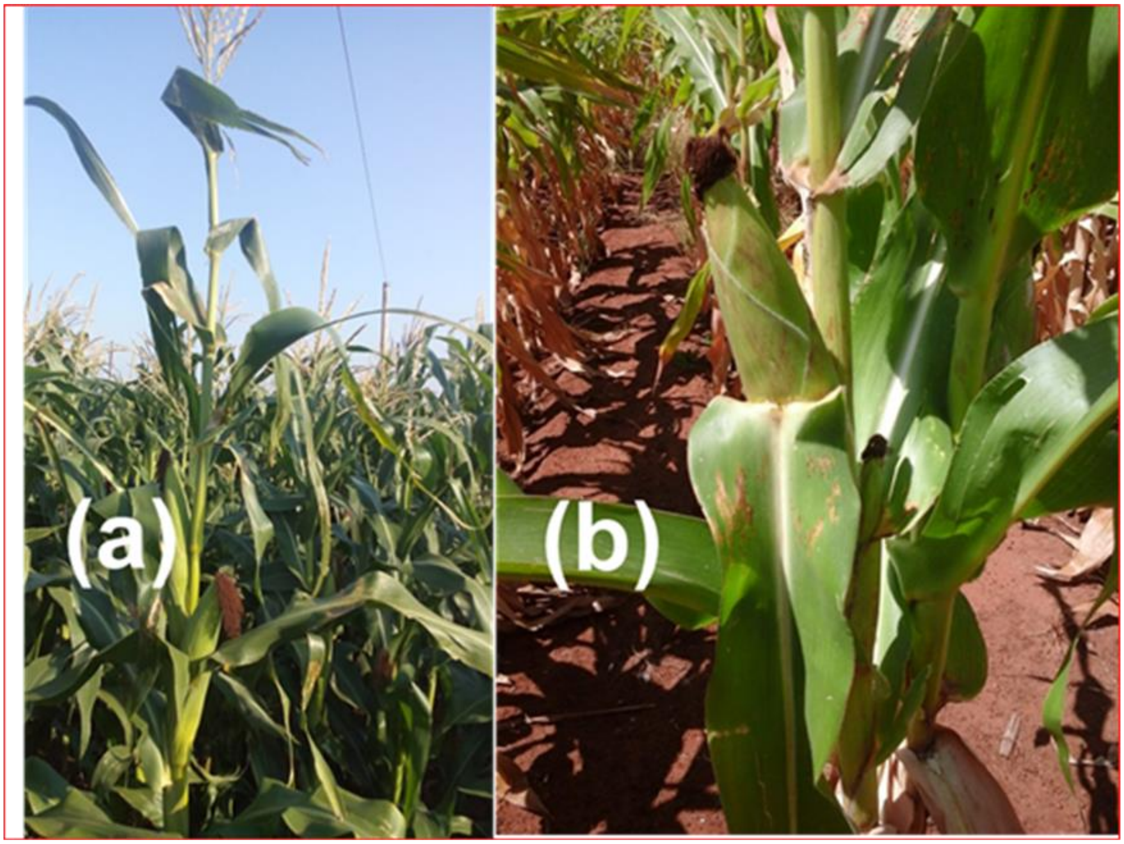

Fig. 4.2 Prolific hybrids under (a) optimum and (b) low N conditions, Potchefstroom, 2015.

Fig. 4.3 Extra but inferior ears were evident in some prolific hybrids.

Fig. 4.4 Some hybrids showed diminished ear sizes and poor seed set under low N.

4.2 Response of prolific hybrids to optimum nitrogen conditions

The analysis of variance (ANOVA) on the performance of the 102 hybrids showed that the general combining ability due to males (GCA_m) was highly significant ($P < 0.01$) at both optimum environments at Potchefstroom and Cedara (Table 4.1). However, the GCA due to females (GCA_f) was significant at Potchefstroom but not at Cedara. At Potchefstroom, the SCA values were lower than the GCA values but the GCA_m effects were more than double the GCA_f effects. The GCA_m contributed 31 % of the total variance for ear prolificacy while the GCA_f contributed only 23 %.

4.2.1 General combining ability of ear prolific hybrids under optimum environments

There were no significant differences among GCA estimates for male (Table 4.2) and female (Table 4.3) inbred lines for ear prolificacy evaluated under optimum conditions at Potchefstroom. The inbred line TZEI63 ranked first as a good male combiner for ear prolificacy followed by T11162W with a GCA estimate of 1.15 at Potchefstroom. The GCA estimates were positive and thus desirable in selecting for prolificacy. CML 538 had a GCA estimate of 0.99 and was The inbred line L15 which was the first ranked female combiner at Potchefstroom for prolificacy (Table 4.3), although it was not significantly different ($P < 0.05$) from the other 12 female inbred lines (rank 13 to 24) (Table 4.3). The inbred lines L6 and L16 (with GCA estimates of 0.83) ranked poorly as female combiners for prolificacy. At Cedara, TZEI63 showed consistency, ranking first as a good male inbred line combiner for ear prolificacy (Table 4.4). Inbred line L17 ranked first as a good female combiner for prolificacy. However, it was not significantly different ($P < 0.05$) from other female combiners (Table 4.5). All GCA estimates for both optimum locations were positive.

Table 4.1 Mean squares from analysis of variance for the number of ears per plant under optimum conditions during the 2014/2015 season at two testing locations.

Source of variation	**df**	**Potchefstroom**	**Cedara**
Block	41	0.08**	0.07**
GCAm	22	0.05**	0.11**
GCAf	23	0.03*	0.04
SCA/location	56	0.02*	0.04*
Error/location		0.02	0.02

**; * Significant at the 1.0 % and 5.0 % probability level, respectively

GCA_m = general combining ability due to males

GCA_f = general combining ability due to females

Table 4.2 General combining ability for ear prolificacy of male inbred lines evaluated under optimum conditions at Potchefstroom.

Rank	**Male**	**GCA estimate**
1	TZEI63	1.15a
2	T1162W	1.14a
3	TZEI56	1.14a
4	RO549W	1.09a
5	CB255	1.08a
6	I-35	1.08a
7	CB232	1.07a
8	CB299	1.05a
9	TZEI11	1.04a
10	CML546	1.03a
11	I-20	1.03a
12	RO452W	1.03a
13	CML489	1.02a
14	TZEI14	1.02a
15	CML538	0.99a
16	CML545	0.99a
17	RO544W	0.98a
18	M37W	0.97a
19	TZEI13	0.95a
20	J80W	0.94a
21	CML521	0.92a
22	TZEI5	0.86a
23	I-34	0.83a
$LSD_{0.05}$		0.75

Means followed by the same letter are not significantly different at the 5.0% probability level.

Table 4.3 General combining ability for ear prolificacy of female inbred lines evaluated under optimum conditions at Potchefstroom.

Rank	**Female**	**GCA estimate**
1	L15	1.21a
2	L1	1.19a
3	L24	1.15a
4	L4	1.13a
5	L13	1.11a
6	L17	1.11a
7	L9	1.08a
8	L8	1.07a
9	L22	1.05a
10	L18	1.03a
11	L21	1.03a
12	L10	1.02a
13	L11	0.98a
14	L12	0.98a
15	L20	0.98a
16	L3	0.98a
17	L2	0.96a
18	L14	0.95a
19	L19	0.95a
20	L23	0.95a
21	L5	0.92a
22	L7	0.89a
23	L6	0.88a
24	L16	0.83a
$LSD_{0.05}$		0.84

Means followed by the same letter are not significantly different at the 5.0% probability level.

Table 4.4 General combining ability for ear prolificacy of male inbred lines evaluated under optimum conditions at Cedara

Rank	Male	GCA estimate
1	TZEI63	1.49a
2	TZEI56	1.40ab
3	RO452W	1.30bc
4	CML546	1.20bc
5	I-35	1.20bc
6	CB255	1.19bc
7	RO549W	1.18bcd
8	I-34	1.15cde
9	T1162W	1.14cde
10	CML489	1.13cdef
11	I-20	1.13cdef
12	CB232	1.11cdefg
13	CB299	1.09cdefg
14	CML545	1.07cdefg
15	M37W	1.07cdefg
16	TZEI13	1.07cdefg
17	TZEI5	1.06cdefg
18	CML538	1.03defg
19	TZEI14	1.03defg
20	RO544W	1.02defg
21	TZEI11	1.01defg
22	J80W	0.99efg
23	CML521	0.98efg
$LSD_{0.05}$		0.14

Means followed by the same letter are not significantly different at the 5.0% probability level.

Table 4.5 General combining ability for ear prolificacy of female inbred lines evaluated under optimum conditions at Cedara.

Rank	Female	GCA estimate
1	L17	1.35a
2	L13	1.33a
3	L24	1.30a
4	L23	1.25a
5	L15	1.23a
6	L12	1.23a
7	L4	1.20a
8	L22	1.18a
9	L18	1.18a
10	L9	1.17a
11	L1	1.15a
12	L10	1.12a
13	L14	1.10a
14	L6	1.10a
15	L20	1.09a
16	L2	1.08a
17	L11	1.08a
18	L3	1.07a
19	L21	1.06a
20	L7	1.06a
21	L16	1.05a
22	L5	1.04a
23	L8	1.02a
24	L19	0.93a
$LSD_{0.05}$		1.16

Means followed by the same letter are not significantly different at the 5.0% probability level.

4.2.2 Specific combining ability of ear prolific hybrids under optimum environments

At Potchefstroom, two hybrids (L1 x R0549W and L15 x TZEI56) produced significantly ($P < 0.05$) high SCA values (1.50) (Table 4.6). However, when L1 was combined with other parental inbred lines (I-20; TZEI14; CML 489 and T1162W), it produced significantly ($P < 0.05$) lower SCA values (Table 4.6). Similarly, the inbred female line L15 showed a significantly ($P < 0.05$) lower SCA estimate (1.20) when combined with the male parent CML 538 (Table 4.6). At least 25 hybrid combinations involving local (ARC) germplasm showed evidence of ear prolificacy. In the trial conducted at Cedara under optimum conditions, the hybrid between L13 x TZEI63 produced the highest (1.95) SCA estimate while ten hybrids produced significantly ($P < 0.05$) lower SCA (1.05) estimates (Table 4.7). Two other hybrids namely L17 x RO452W and L15 x TZEI56 also produced relatively high (1.70) SCA estimates.

4.2.3 General and specific combining ability ratio

Overall, the GCA:SCA ratios for NEP under optimum and low N conditions was 1:2 and 1:1, respectively. In addition, the GCA:SCA ratio for grain yield under both conditions was 1:1.

Table 4.6 Estimates of SCA for ear prolificacy of hybrids (producing >1 cob) evaluated under optimum conditions at Potchefstroom.

Rank	Hybrid combination		SCA Estimate	Rank	Hybrid combination		SCA Estimate
	Male	**Female**			**Male**	**Female**	
1	RO549W	L1	1.50a	24	I-35	L10	1.13bcdef
2	TZEI56	L15	1.50a	25	CML538	L14	1.12bcdef
3	T1162W	L4	1.40ab	26	CML489	L1	1.11cdef
4	CB255	L15	1.35bc	27	CB255	L13	1.10cdef
5	I-35	L9	1.30bc	28	CB232	L10	1.10cdef
6	CB299	L15	1.25bcd	29	CB232	L8	1.05def
7	T1162W	L21	1.25bcd	30	CB232	L9	1.05def
8	TZEI11	L7	1.25bcd	31	CML538	L13	1.05def
9	CML538	L15	1.20bcd	32	CML545	L8	1.05def
10	RO452W	L17	1.20bcde	33	CML545	L9	1.05def
11	TZEI56	L17	1.16bcdef	34	CML546	L20	1.05def
12	CB232	L12	1.15bcdef	35	M37W	L14	1.05def
13	CB299	L17	1.15bcdef	36	RO544W	L18	1.05def
14	I-20	L1	1.15bcdef	37	RO549W	L21	1.05def
15	I-20	L4	1.15bcdef	38	RO549W	L22	1.05def
16	TZEI14	L1	1.15bcdef	39	T1162W	L1	1.05def
17	TZEI5	L13	1.15bcdef	40	T1162W	L3	1.05def
18	TZEI56	L24	1.15bcdef	41	TZEI11	L5	1.05def
19	TZEI63	L11	1.15bcdef	42	TZEI13	L4	1.05def
20	TZEI63	L13	1.15bcdef	43	TZEI13	L6	1.05def
21	T1162W	L22	1.15bcdef	44	TZEI14	L2	1.05def
22	CHECK3	CHECK3	1.15bcdef	45	TZEI5	L12	1.05def
23	I-35	L8	1.14bcdef	-	-	-	-
$LSD_{0.05}$			0.13				0.13

Means followed by the same letter are not significantly different at the 5.0% probability level.

Table 4.7 Estimates of SCA for ear prolificacy of hybrids (producing >1 cob) evaluated under optimum conditions at Cedara.

Rank	Hybrid combination		SCA Estimate	Rank	Hybrid combination		SCA Estimate
	Male	Female			Male	Female	
1	TZEI63	L13	1.95a	35	T1162W	L21	1.15bcdef
2	RO452W	L17	1.70ab	36	TZEI13	L3	1.15bcdef
3	TZEI56	L15	1.70ab	37	TZEI13	L6	1.15bcdef
4	TZEI63	L12	1.55bc	38	TZEI56	L14	1.15bcdef
5	CB232	L12	1.50bcd	39	CB232	L10	1.10bcdef
6	I-34	L5	1.45bcde	40	CB255	L13	1.10bcdef
7	TZEI56	L17	1.45bcde	41	CB255	L14	1.10bcdef
8	TZEI63	L11	1.45bcde	42	CB299	L14	1.10bcdef
9	I-35	L9	1.35bcde	43	CML489	L2	1.10bcdef
10	TZEI5	L13	1.35bcde	44	CML546	L17	1.10bcdef
11	CB255	L15	1.30bcdef	45	I-34	L2	1.10bcdef
12	CML546	L20	1.30bcdef	46	J80W	L9	1.10bcdef
13	I-20	L4	1.30bcdef	47	M37W	L14	1.10bcdef
14	I-35	L10	1.30bcdef	48	M37W	L16	1.10bcdef
15	RO549W	L1	1.30bcdef	49	T1162W	L2	1.10bcdef
16	RO549W	L18	1.30bcdef	50	T1162W	L3	1.10bcdef
17	RO549W	L21	1.30bcdef	51	TZEI11	L7	1.10bcdef
18	T1162W	L22	1.30bcdef	52	TZEI13	L4	1.10bcdef
19	TZEI56	L24	1.30bcdef	53	TZEI14	L2	1.10bcdef
20	CB255	L12	1.25bcdef	54	TZEI14	L22	1.10bcdef
21	CML489	L1	1.25bcdef	55	TZEI5	L12	1.10bcdef
22	CML538	L15	1.25bcdef	56	TZEI5	L14	1.10bcdef
23	CML545	L9	1.25bcdef	57	TZEI5	L16	1.10bcdef
24	I-35	L7	1.25bcdef	58	CB232	L8	1.05bcdef
25	RO452W	L23	1.25bcdef	59	CML489	L3	1.05bcdef
26	CML521	L20	1.20bcdef	60	CML538	L14	1.05bcdef
27	I-20	L1	1.20bcdef	61	CML538	L16	1.05bcdef
28	I-34	L4	1.20bcdef	62	CHECK3	CHECK3	1.05bcdef
29	T1162W	L4	1.20bcdef	63	I-34	L6	1.05bcdef
30	CB299	L15	1.15bcdef	64	I-35	L8	1.05bcdef
31	CB299	L17	1.15bcdef	65	J80W	L7	1.05bcdef
32	CML545	L10	1.15bcdef	66	RO544W	L18	1.05bcdef
33	I-34	L3	1.15bcdef	67	TZEI11	L10	1.05bcdef
34	RO549W	L22	1.15bcdef	68	TZEI13	L7	1.05bcdef
$LSD_{0.05}$							0.29

Means followed by the same letter are not significantly different at the 5.0% probability level.

4.3 Grain yield performance of prolific hybrids

There were highly significant ($P \leq 0.01$) differences in grain yield for both GCA_m and GCA_f at Potchefstroom (Table 4.8). Similarly, there were significant ($P \leq 0.05$) differences in grain yield for GCA_m but not for GCA_f at Taung. The effects of the N stress conditions that were imposed on the trial at Taung were already showing at the 8th leaf stage when compared to hybrids evaluated in Potchefstroom low N conditions (Fig. 4.5).

The highest grain yield (2.91 t/ha) at Potchefstroom was attained by the hybrid between L20 x CML521 (Table 4.9). In contrast, he highest grain yield (1.92 t/ha) at Taung was attained by the hybrid between L8 x CB232. Under the optimum conditions at Potchefstroom, at least 10 hybrids achieved > 2.50 t/ha (Fig. 4.6). However, only two of the best 10 hybrids achieved > 1.00 t/ha under the low N conditions that were imposed at Taung (Fig. 4.6). Nonetheless, four other combinations that were not ranked among the best 10 attained > 1.00 t/ha (Table 4.9). The ear size was generally reduced under low N stress conditions in comparison with the performance under optimum conditions in which four of the best performing genotypes obtained > 5.00 t/ha (Appendix I).

Table 4.8 Mean squares for the analysis of variance for yield under low N conditions during the 2014/2015 season.

Source of variation	df	Potchefstroom	Taung
Block	41	0.77**	0.31*
GCAm	22	0.57**	0.17*
GCAf	23	0.35**	0.21
SCA/set	56	0.38**	0.14*
Error/set		0.11	0.09

**; * Significant at the 1.0 % and 5.0 % probability level, respectively

GCA_m = general combining ability due to males

GCA_f = general combining ability due to females

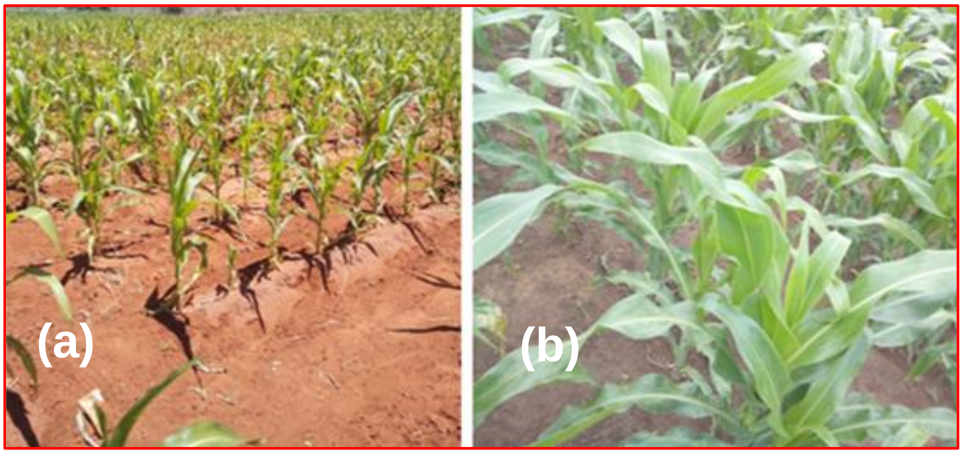

Fig. 4.5 Maize hybrids at 8th leave stage at (a) Taung optimum, (b) Potchefstroom optimum.

Table 4.9 Yield estimates of prolific hybrids evaluated under low nitrogen conditions in Potchefstroom and Taung.

	Hybrid combination		Grain yield (t/ha)			Hybrid combination		Grain yield (t/ha)	
	Male	Female	Potchefstroom	Taung		Male	Female	Potchefstroom	Taung
1	CML521	L20	2.91a	0.82bcde	38	CML538	L16	1.98abcd	0.70bcde
2	TZEI13	L3	2.82ab	0.74bcde	39	TZEI56	L14	1.98abcd	0.48bcde
3	T1162W	L1	2.81ab	0.88bcde	40	CB255	L14	1.96abcd	0.85bcde
4	CB232	L8	2.76abc	1.92a	41	TZEI14	L21	1.96abcd	0.49bcde
5	TZEI56	L15	2.73abcd	0.78bcde	42	TZEI13	L7	1.93abcd	0.48bcde
6	CML538	L14	2.65abcd	1.28bcde	43	I-35	L9	1.91cde	0.82bcde
7	T1162W	L22	2.63abcd	0.72bcde	44	CHECK3	CHECK3	1.90cde	0.76bcde
8	RO452W	L17	2.59abcd	0.90bcde	45	TZEI5	L16	1.90cde	0.98bcde
9	I-20	L4	2.56abcd	0.61bcde	46	TZEI5	L14	1.89cde	0.74bcde
10	I-35	L10	2.51abcd	0.94bcde	47	TZEI63	L11	1.86cde	0.82bcde
11	TZEI63	L12	2.49abcd	0.96bcde	48	TZEI14	L22	1.83cde	0.45bcde
12	TZEI5	L12	2.48abcd	1.02bcde	49	RO549W	L21	1.82cde	0.53bcde
13	RO549W	L22	2.45abcd	0.72bcde	50	M37W	L12	1.79def	0.54bcde
14	CML489	L1	2.38abcd	0.96bcde	51	TZEI14	L1	1.79def	0.19bcde
15	CML546	L20	2.32abcd	0.77bcde	52	I-20	L3	1.74def	0.19bcde
16	TZEI56	L17	2.31abcd	0.76bcde	53	RO549W	L19	1.73def	0.43bcde
17	CML545	L8	2.26abcd	1.69ab	54	J80W	L10	1.72def	1.31bcd
18	RO549W	L20	2.26abcd	0.70bcde	55	TZEI13	L4	1.71def	0.48bcde
19	TZEI14	L2	2.26abcd	1.48abc	56	I-35	L8	1.70def	0.72bcde
20	CB255	L15	2.24abcd	0.95bcde	57	CB232	L10	1.69def	0.50bcde
21	TZEI56	L24	2.24abcd	0.71bcde	58	CB255	L13	1.69def	0.81bcde
22	TZEI11	L8	2.23abcd	0.62bcde	59	CB255	L13	1.69def	0.49bcde
23	T1162W	L2	2.22abcd	0.69bcde	60	CML546	L17	1.69def	0.49bcde
24	CB255	L12	2.18abcd	0.95bcde	61	CML538	L13	1.67def	0.25bcde
25	RO544W	L20	2.15abcd	1.13bcde	62	TZEI5	L13	1.59def	0.52bcde
26	TZEI13	L6	2.15abcd	0.58bcde	63	CB232	L12	1.55def	0.21bcde
27	CML489	L3	2.13abcd	0.29bcde	64	J80W	L9	1.55def	0.41bcde
28	T1162W	L21	2.09abcd	0.66bcde	65	I-35	L7	1.53def	0.34bcde
29	CB299	L17	2.07abcd	0.47bcde	66	TZEI63	L13	1.49def	0.79bcde
30	RO549W	L18	2.07abcd	0.61bcde	67	I-34	L3	1.47def	0.19bcde
31	T1162W	L3	2.07abcd	0.62bcde	68	RO544W	L19	1.47def	0.89bcde
32	I-34	L6	2.05abcd	0.77bcde	69	RO452W	L23	1.41def	0.56bcde
33	CML538	L15	2.02abcd	1.06bcde	70	CML545	L5	1.39def	0.71bcde
34	I-20	L2	2.02abcd	0.28bcde	71	M37W	L14	1.38def	0.19bcde
35	I-34	L4	2.01abcd	0.71bcde	72	RO544W	L18	1.38def	0.69bcde
36	T1162W	L4	1.99abcd	0.33bcde		-	-	-	-
$LSD_{0.05}$			0.67	0.59				0.67	0.59

Means followed by the same letter are not significantly different at the 5.0% probability level.

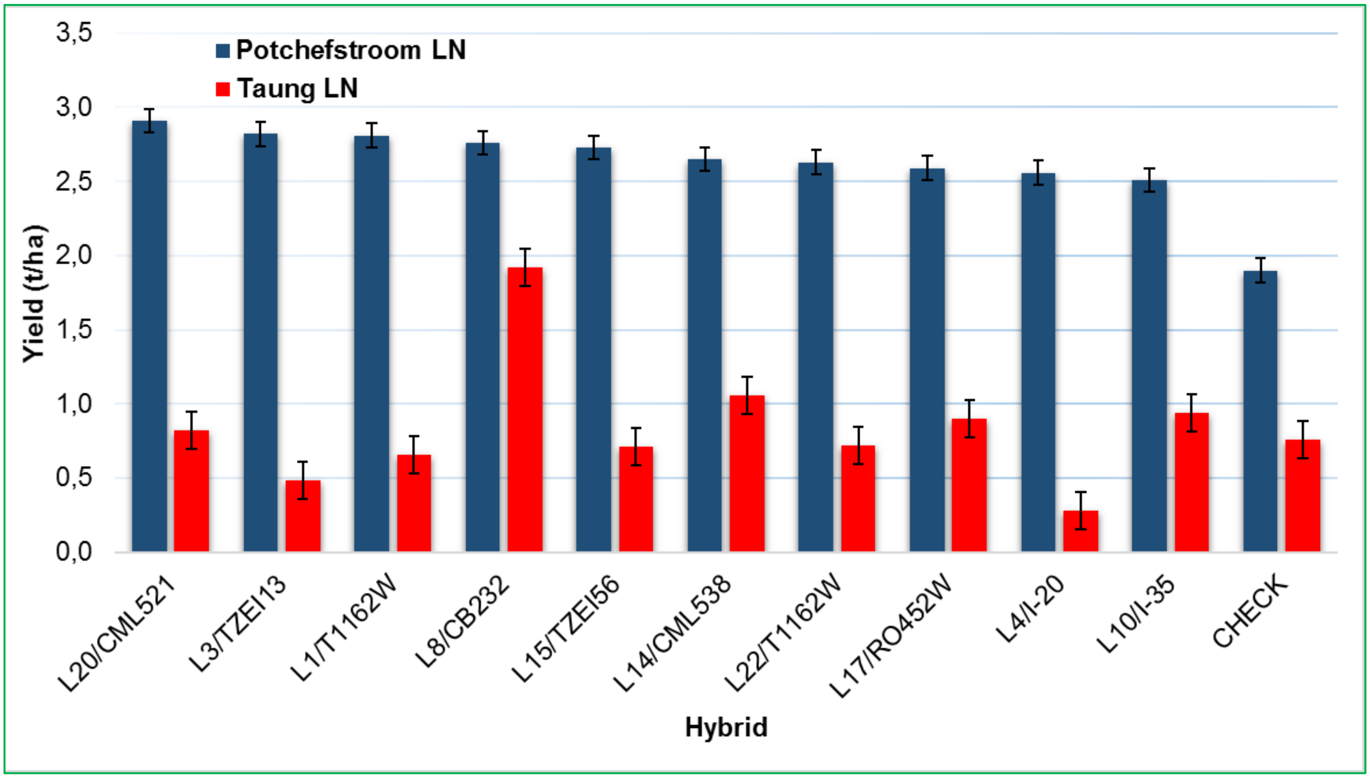

Fig. 4.6 The grain yield of the best ten prolific hybrids under low nitrogen stress conditions.

4. 4 Correlations between agronomic traits among the prolific maize hybrids

Under optimum conditions, there were highly significant ($P < 0.01$) positive correlations between ear diameter (ED) and ear length (EL), ear height (EH) as well as the number of ears per plant (NEP) (Table 4.10). However, there was a negative (- 0.26) but highly significant ($P < 0.01$) correlation between ED and grain yield (GY). In contrast, the NEP showed a highly significant ($P < 0.01$) positive correlation with the GY (Table 4.10). Under low N conditions, the anthesis-silking interval (ASI) showed a negative but highly significant ($P < 0.01$) correlation with each of the traits (Table 4.11).However, the ED showed a positive and highly significant ($P < 0.01$) correlation with each of the traits that was evaluated. Similarly, EL and EH showed positive and highly significant ($P < 0.01$) correlations with each of NEP and GY (Table 4.11).

Table 4.10 Pearson correlation coefficients among prolific maize hybrids evaluated under optimum conditions at two testing locations (Potechefstroom and Cedara).

	ASI	**ED**	**EL**	**EH**	**NEP**	**GY**
ASI	1.00					
ED	-0.12*	1.00				
EL	-0.07	0.28**	1.00			
EH	0.00	0.27**	0.04	1.00		
NEP	0.01	0.17**	0.27**	0.25**	1.00	
GY	0.13*	-0.26**	-0.15**	-0.05	0.18**	1.00

**; * Significant at the 1.0% and 5.0% probability level, respectively.

Table 4.11 Pearson correlation coefficients among prolific maize hybrids evaluated under low nitrogen conditions at two testing locations (Potchefstroom and Cedara).

	ASI	**ED**	**EL**	**EH**	**NEP**	**GY**
ASI	1.00					
ED	-0.25**	1.00				
EL	-0.33**	0.45**	1.00			
EH	-0.31**	0.32**	0.35**	1.00		
NEP	-0.33**	0.42**	0.53**	0.49**	1.00	
GY	-0.44**	0.53**	0.64**	0.52**	0.77**	1.00

**; * Significant at the 1.0% and 5.0% probability level, respectively.

CHAPTER 5: DISCUSSION

In the approach which involved evaluation of hybrids under both optimum and N stressed conditions was able to demonstrate the variation in hybrid performance particularly prolificacy. Under optimum conditions, the results showed that the full potential of prolificacy was expressed with hybrids developing normal ears. In contrast, the low N stress conditions led to some considerable reduction in ear attributes and grain yield. This was consistent with the results reported in a similar study by Shanti *et al.,* (1997). The authors attributed this observation to a reduction of leaf area which in turn negatively impacted on photosynthetic rate. Other studies attributed the reduction in ear attributes and grain yield under low N conditions to reduced leaf area index, photosynthetic rate and light interception which consequently reduced biomass production, kernel number and grain yield (Uhart and Andrade, 1995a, b). On the other hand, other researchers cited the interference in metabolic activities (Biswas and Mukherjee, 1993) as well as chlorophyll degradation (Buchanan-Wollaston *et al.*, 2003).

Although the combining ability approach was used in maize to evaluate gene action, it was widely used also in sunflower (Machikowa *et al*., 2011), sorghum (Makanda *et al.,* 2010) and other crops. Therefore the methodologies used in this study was consistent with the approach used by other researchers to determine the gene action of prolificacy in maize. The GCA and SCA values were within the range that was reported in similar studies previously (Afekhai *et al.*, 2017; Murtadha and Alghamdi, 2018). The GCA and SCA estimates for NEP in this study were positive indicating dominance and additive gene action in the germplasm pool for ear prolificacy. This particularly implies that special attention needs to be paid to in breeding for stress tolerance since it is one of the major component of grain yield in maize (Betrán *et al*., 2003). Although hybrids were subjected to stress, an expression of positive estimates for NEP were also reported (Al-Naggar *et al*., 2015). Significant general combining ability estimates for inbred lines have also been reported (Aguia *et al.,* 2003). However, these studies utilized the diallel mating design as compared to this study.

Positive combining ability estimates for yield and NEP are desirable (Akinwale *et al.* 2014; Bertoia and Aulicino, 2014; Ketthaisong *et al.*, 2014). This is an indication that the germplasm was of good genetic value and the associated genes could provide a breakthrough to stress tolerance breeding (Betrán *et al*., 2003). In contrast, negative combining ability estimates for grain yield in maize have also been observed in other studies (Fan *et al., 2018;* Zhang *et al., 2017*). The negative estimates would mean that the germplasm pool was narrow for hybrid development (Hoecker *et al.,* 2005; Muraya *et al., 2006*).

In another study involving wheat, the variance ratio for GCA was higher than that for SCA indicating higher values of additive versus dominance effects (Gowda *et al.,* 2012). The authors asserted that the findings were useful in selection based on GCA effects in wheat which is also a cereal crop (Borghi and Perenzin, 1994; Perenzin *et al.*, 1998). Although the gene action for grain yield did not fully indicate the predominance of GCA/SCA gene action, NEP gene action was fully expressed by the genotypes. Nonetheless, this may be worthwhile applying also in maize breeding programmes, hence the results reported in this study will contribute to the breeding of prolificacy in maize. However, genetic variance ratios are often too low across environments thus making it difficult to draw valid conclusions about gene action for a specific trait (Talabi *et al.,* 2017). Although the gene action for grain yield did not fully indicate the predominance of GCA/SCA gene action, NEP gene action was fully expressed by the genotypes.

The results of the study that were reported in this document also clearly indicated that specific parental combinations produced prolific hybrids. In particular, the germplasm from IITA carried the prolificacy trait which was expressed even under low N conditions. The hybrids developed between the IITA and local germplasm displayed this desirable trait thus suggesting that it is feasible to create prolific maize hybrids for the low N conditions that are present in South Africa. The results were in agreement with the observations that were reported previously in numerous studies (Mueller and Vyn, 2016). However, some of the hybrids in this study showed low seed set and diminished cob sizes indicating that ear prolificacy should be evaluated together with the realized grain yield of the prolific hybrids. In practical terms, the farmers are interested in the productivity of the hybrid, prolific or otherwise. Nonetheless, the prolific hybrids that were evaluated in the study indicated a reasonable grain yield potential under optimum conditions, attaining up to three-fold higher yield than under low conditions. This suggested that probably with better farmer management under low N conditions, higher grain yields could be attained. In general, higher maize yields reaching up to 8.0 t/ha (Sapkota *et al.,* 2017) have been attained. The relatively low grain yields of these hybrids could be threatened even more if the crop is subjected to a combination of low and moisture stress (Talabi *et al., 2017)*. Therefore, in situations that are prone to moisture stress as well as low N, the evaluation of potential prolific hybrids can consider the two factors.

The performance of the hybrids was evaluated over a limited number of environments and seasons. The significant difference between blocks also indicated the presence of non-uniformity and can also be attributed to external factors. Increasing the number of replications can also be beneficial in order to reduce error. Previous studies utilized multiple environments and increased replications in order to obtain more accurate results particularly the stability of hybrids (Tollenaar and Lee, 2002). In the case of local environments, apart from utilizing the test locations in North West Province only, there will be merit in expanding the number of test locations to other low N areas in other Provinces such as Limpopo Eastern Cape. For instance, Odhiambo *et al*., (2011) reported that availability of chemical nitrogenous fertilizer was one of the major constraints in maize production in Limpopo Province. Similarly, smallholder maize growers in other countries in the region were faced with limited availability of inputs including nitrogenous fertilizer (Kihara *et al., 2016*). In another study which used a survey approach in Kenya, farmers also indicated the importance of chemical nitrogenous fertilizer in smallholder production systems (Ribeiro *et al.,* 2017).

The positive phenotypic correlation between grain yield and number of ears per plant at both optimum and low N stress conditions was consistent with the findings reported previously elsewhere (Abadasi, 2017). In addition, the negative correlation between anthesis silking interval (ASI) and grain yield was widely reported in other studies (Dari *et al., 2016*). This negative relationship is desirable particularly under abiotic stress conditions (Bänziger *et al.,* 2006) since early maturing hybrids can have a better chance escaping particularly terminal season drought. Gallais and Coque (2005) also reported that prolific hybrids with a short ASI have the advantage to remobilise N from stover left from the previous season and thus aiding the plant with embryo development. This further avoids occurrence of extreme kernel and ear abortion which drastically reduce yields. It is evident that the germplasm used for the study has potential for early maturing hybrids that can utilise N efficiently while optimising grain yields. A negative correlation between ear length (EL) and ear diameter (ED) to grain yield which was observed under optimum conditions in this study, was also reported previously (Bahoush and Abbasdokht, 2008). This suggested that, both EL and ED do not contribute significantly to grain yield unlike under stress conditions. Increased diameter and length of the ears promotes kernel development and thus increasing grain yield (Otegui and Bonhomme, 1998).

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

The combining ability approach is an essential tool in stress breeding. In order to advance breeding programmes, the use of exotic sources together with local genotypes to create specific hybrids that are tolerant to stress is of utmost importance. The exotic source of germplasm introduced in this study contributed significantly to the expression of prolificacy. Prolificacy was also evident in some hybrids even under these conditions. Although there were no significant differences among both male and female inbred lines, their positive general combining ability estimates suggest presence of the prolificacy trait in both the exotic and local germplasm. This further suggests that, these inbred lines can be used as a genetic pool for breeding that is aimed at increasing yields through prolificacy.

Under low N, yield was substantially lower suggesting that more crosses will be necessary to produce more productive hybrids. Breeders should consider using prolific type inbred lines when breeding for stress tolerance as a combination of inbred lines with such traits gives at least one cob. The use of exotic sources of germplasm will diversify local germplasm thus improving hybrids that are recommended for framers.

Although yields in the environment represented by the test location at Taung were lower, some of these hybrids developed can be evaluated further in this area as it consists of many smallholder farmers that still face low N fertility constraints. It would be interesting to consider other essential traits of the germplasm such as protein and oil (Appendix I). These are important traits for framers that rely on the maize crop as a source of their nutrients.

The study showed that grain yield was consistently correlated positively and significantly with the number of ears per plant. This suggested that it is an essential trait to consider in a maize breeding programmes aimed at the improvement of grain yield. The results suggested that there will be merit in focusing on prolific germplasm in breeding for low N conditions similar to Taung. The prolific hybrids produced at least one cob under low N conditions. The involvement of a wide range of prolific germplasm could be useful for the development of more prolific hybrids for the low N areas.

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APPENDICES

APPENDIX I

Research Notes

Department of Plant Production, School of Agriculture, University of Venda, March 26, 2018

A Preliminary Survey of the Grain Yield Potential, Protein and Oil Content in Maize Prolific Hybrids Raised Under Optimum Conditions

[1]Makhumbila P., [2]Gwata, E.T. and [1]Mashingaidze K.

[1]Grain Crops Institute – ARC, Private Bag X1251, Potchefstroom 2520, South Africa

[2]Department of Plant Production, University of Venda, P. Bag X5050, Thohoyandou 0950, South Africa

(Extended Abstract for a Local Citation; Unpublished)

1.0 Introduction

Maize (*Zea mays*) is a staple food crop for many people in Africa. Worldwide, the crop is produced largely from hybrid seed in order to optimize production per unit area. In addition, prolific hybrids tend to produce more grain yield than non-prolific hybrids (Mendes-Moreira *et al*., 2014; Varga *et al*., 2004; Sarquis *et al*., 1998; Priorand Russell, 1975).In South Africa, growers generally use single cross hybrid varieties in order to optimize yields across diverse agro-ecological zones. However, the determination of the grain yield potential, protein and oil content of prolific varieties has not been investigated adequately. Therefore, this study was designed to evaluate selected maize hybrids for grain yield potential, percent kernel protein and oil content.

2.0 Materials and methods

A diverse set of 70 single-cross hybrids (including a check) was evaluated in the field at two testing locations (Potchefstroom, North West Province and Cedara, Kwa-Zulu Natal Province, South Africa) using a 0.1 alpha lattice experimental design (Makumbila *et al*., 2016) replicated twice during the 2014/2015 summer season. The seed of each hybrid was planted in a field plot consisting of two rows 4.0 m long, spaced 0.9 m apart. In each row, the plants were spaced at 0.12 m from each other. Basal fertilizer was applied at planting at the rate of 40.0kg/ha (du Plessis, 2003).At maturity, grain yield was measured and five plants were selected randomly from each plot and used for determining the kernel protein and oil content using the electromagnetic resonance method (Hymowitz *et al*., 1974).

3.0 Results and Discussion

There were highly significant (P<0.01) differences in grain yield among the hybrid varieties. The highest grain yield (7.7 t/ha) was observed for hybrid 'L14 x CML 538' at Cedara (Fig. A-1). This grain yield was >20.0% lower than the grain yield (10.4 t/ha) reported previously in similar studies (Varga *et al*., 2004).The results also showed that there were significant (P<0.05) differences in both percent protein and percent oil among the maize hybrids. The percent protein ranged from 5.4 to 12.1% but the percent oil ranged between 4.2 – 5.95%. The highest percent protein was observed for the hybrid 'L15 x TZEI5' and the best (Fig. A-2). On average, the hybrid varieties attained 5.00 % oil (Fig. A-3). A poor negative relationship was observed between the percent protein and percent oil (Fig. A-4). This was in agreement with findings from similar studies involving maize reported previously (Ilyas *et al*., 2014; Abou-Deif *et al*., 2012; Seiam and Khalifa, 2007). The wide variation % protein suggested that there was potential for selecting for this trait. However, multiple test locations and seasons would be necessary in order to validate the stability of the superior hybrids particularly in grain yield.

4.0 Conclusion

The results showed that the prolific hybrids could produce fairly moderate grain yields under the prevailing agro-ecological conditions. The results also indicated a wider variation for percent kernel protein than for percent kernel oil. In future, the hybrids can be evaluated in more environments and over many seasons in order to validate their stability particularly in terms of grain yield.

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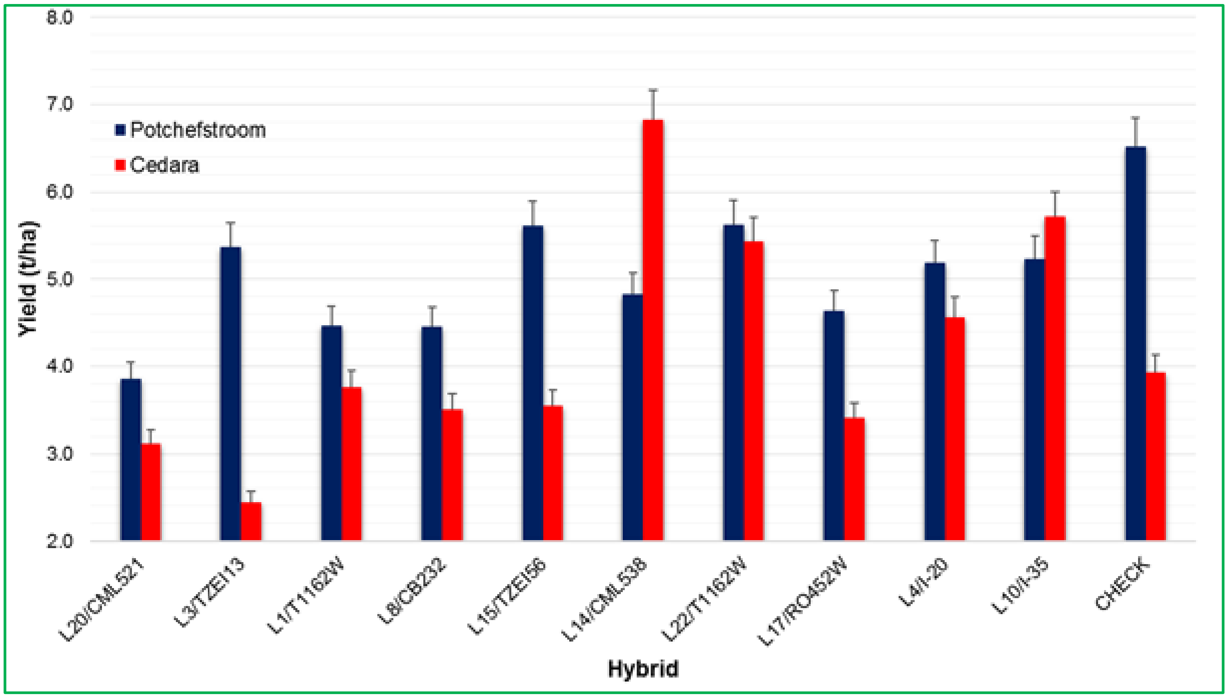

Fig. A-1 Grain yield of the top 10 prolific maize hybrids under optimum conditions at two testing locations.

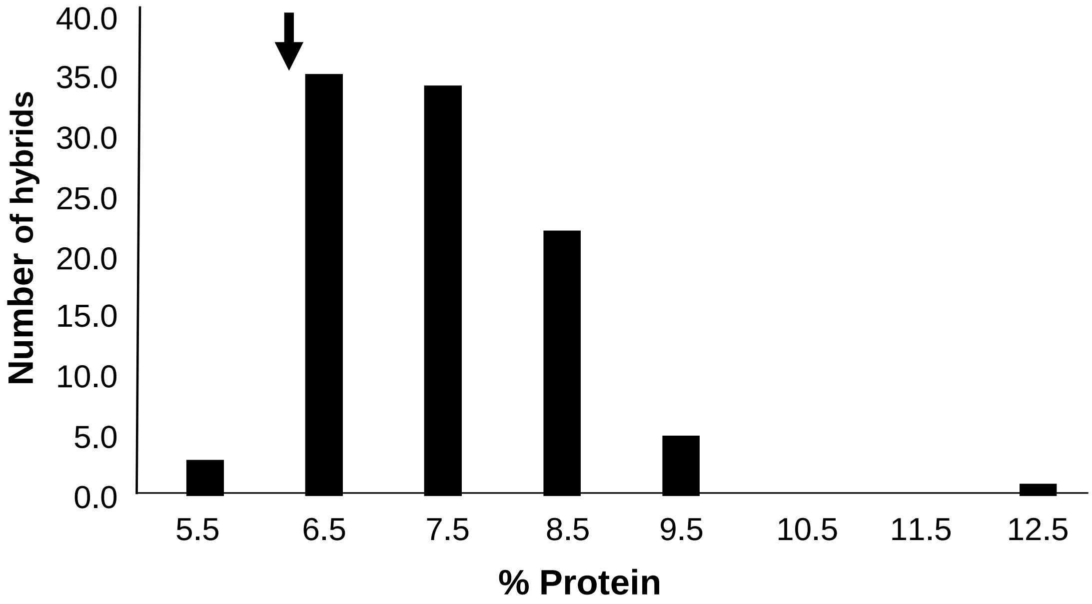

Fig. A-2 Distribution of % kernel protein among selected single cross maize hybrids evaluated during the 2015/16 cropping season. (Arrow indicates approximate mean % protein of the check).

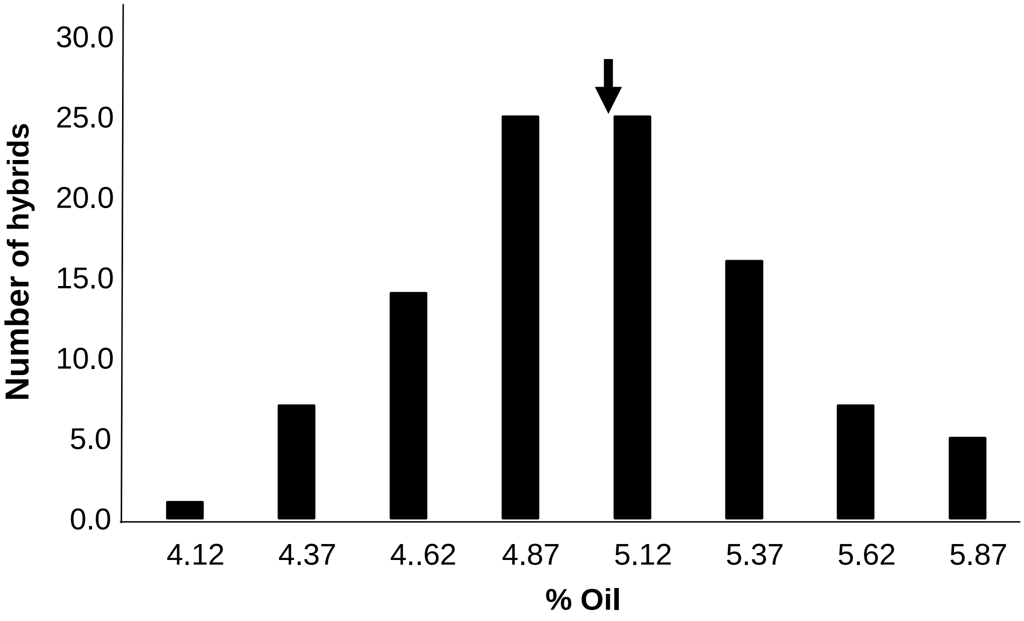

Fig. A-3 Distribution of % kernel oil among selected single cross maize hybrids evaluated during the 2015/16 cropping season. (Arrow indicates approximate mean % oil of the check).

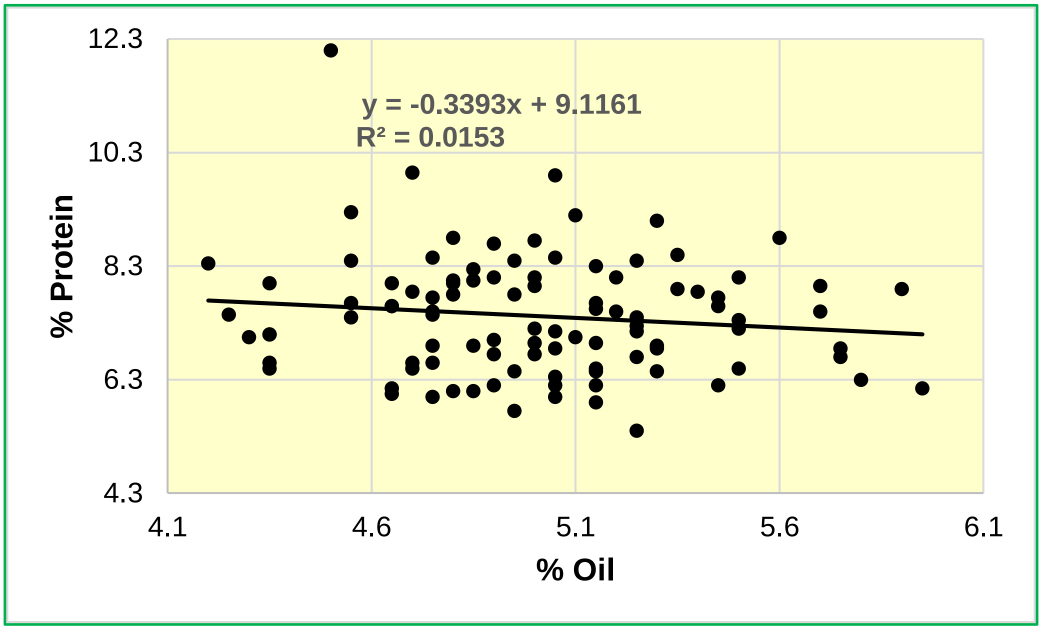

Fig. A-4 The relationship between % protein and % oil among selected single cross maize hybrids evaluated during the 2015/16 cropping season.