

**Heterosis, Genetic Distance and Path Coefficient Analysis
in Dent, Flint and Popcorn Hybrids**

By

Lorraine Mhoswa

BSc. Hort (Hons) (Midlands State University - Zimbabwe)

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School of Agricultural, Earth and Environmental Sciences

College of Agriculture, Engineering and Science

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GENERAL ABSTRACT

Maize (*Zea mays* L.) is one of the most important food crops in sub-Saharan Africa (SSA); however its production is constrained by many factors. Grain yield is compromised by poor genetic performance and poor agronomic management. This calls for need to develop hybrids and exploiting heterosis of single crosses which are adapted to challenging environments. Currently, there is no popcorn hybrids developed in South Africa which is adapted to local conditions. As such, there is need to develop hybrids that cater for small-scale farmers in marginal environments. The objectives of the study were to determine i) standard heterosis, levels of variation and heritability for phenotypic traits in dent and flint maize hybrids; ii) the association between genetic distances and phenotypic traits in dent and flint maize hybrids; iii) mid-parent heterosis in popcorn hybrids, iv) the effect of secondary traits on grain yield in dent, flint and popcorn hybrids; v) genetic diversity and the relationship between traits in widely grown selected hybrids in Southern Africa; and vii) to compare effectiveness of phenotypic analysis models for determining genetic distances between hybrids. Popcorn, dent and flint hybrids were evaluated at two sites. The data was analysed using SAS, Genstat and Power marker statistical packages. The results revealed that the relationship between genetic distance and heterosis is dependent on the environment. Hybrids in top 10 at both sites were different indicating that there was a significant genotype x environment interaction. 13 new heterotic patterns that performed better than the controls can be utilized in heterosis breeding; however there is need to test them in different environments to check on their stability. Grain texture cannot be used to discriminate hybrids for yield because all patterns of dent x dent, dent x flint and flint x flint were present in the top 10 hybrids. Lines DXL124 and DXL158 dominated parentage of the top 10 hybrid rank for yield qualifying them as potential testers for specific combining ability in future studies. Heterosis in popcorn hybrids that performed better than the mid-parent can be utilized in heterosis breeding to exploit vigour, though there is need to test the hybrids in a number of different environments. The main direct factors contributing to yield were ear prolificacy, ear aspect, number of plants and shelling percentages qualifying them to be selected to boost grain yield. Phenotypic data and 91 SNP markers were used to estimate the genetic distance between the hybrids. The results indicated that hybrids that were in the same cluster belong to the same brand and were related in origin and pedigree. Both molecular and phenotypic data were effective in discriminating the hybrids into different clusters according to genetic background. SNP markers revealed nine clusters of hybrids, 12-trait model revealed eight clusters and five-trait model revealed six clusters at 85% genetic distance. The study indicates strategies that can be adopted to boost grain yield in dent, flint and popcorn hybrids.

DECLARATION

I, Lorraine Mhoswa, declare that:

1. The research reported in this dissertation, except where otherwise indicated, and is my original research.
2. This dissertation has not been submitted for any degree examination at any other university.
3. This dissertation does not contain other person's data, picture, graphs or other information, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted. Then:
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Signed

.....
Lorraine Mhoswa

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

.....

Prof. John Derera (Main-supervisor)

.....

Dr. Alfred Odindo (Co-supervisor)

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DEDICATION

To almighty God, who made everything possible and carried me through all the challenges. This piece of work is dedicated to my husband Peter Maruve, my father Karrah-Moyo Mhoswa, mother Musodzi Madiri and my lovely son Kunaishe.

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LIST OF ABBREVIATIONS

AD = anthesis date

AFLP = Amplified Fragment Length Polymorphism

cm = centimetre

CV = Coefficient of variation

$^{\circ}\text{C}$ = degree Celsius

DNA = Deoxyribonucleic acid

FAOSTAT = Food and Agriculture Organisation Statistics

F1 = first filial generation

GD = genetic distance

GCV = genotypic coefficient variation

GEI = genotype by environment interaction

H^2 = broad sense heritability

IMRD = Introduction, Materials and Methods, Results and Discussion.

kg = kilogram

m.a.s.l = meters above sea level

mm = millimetre

MT = Metric tons

PCV = phenotypic coefficient variation

R^2 = Coefficient of determination

RFLP = Restriction Fragment Length Polymorphism

SAS = Statistical Analysis Software

SD= silking date

SNP = Single Nucleotide Polymorphism

SSA = Sub-Saharan Africa

SSR = Simple Sequence Repeats

StdHET = standard heterosis

t ha⁻¹ = tonnes per hectare

% = percentage

INTRODUCTION TO DISSERTATION

Importance of Maize

Maize (*Zea mays* L.) is one of the most important food crops and commonly grown in all parts of sub-Saharan Africa (SSA). Maize is the most consumed cereal in SSA hence it impacts on food security (Heisey and Edmeades, 1999). Maize is grown in a wide range of ecological environments and it is used as food for human beings, while in developed countries it is also used as livestock feed (M'mboyi et al., 2010). Maize is also used for feed in developing countries. For instance, yellow maize is grown for feed in South Africa (Miene and Flourie, 2013). Maize accounts for an average of 32% of consumed calories in Eastern and Southern Africa (Heisey and Edmeades, 1999).

Maize contributes 15-50% of energy in most human diets in SSA (Kagoda et al., 2009). This reflects its role as the primary staple food for the majority of the population (Magorokosho, 2006). Maize yields remain low across SSA at 1.6 t ha^{-1} , only just enough to reach self-sufficiency in many areas (FAOSTAT, 2010). Global cereal demand in 2020 is estimated at 2.1 billion metric tonnes and maize is estimated at 852 million metric tonnes, in SSA the demand is estimated at 52 million metric tonnes (FAOSTAT, 2013). Maize yields are extremely low, yet maize is critical for food security in some of the world's poorest regions in Africa, Asia, and Latin America (Shiferaw et al., 2011). United States of America is the world's largest producer (Figure 1.1) of maize hence they have the highest yields that are close to three times that produced in South Africa (Figure 1.2). Mexico, Argentina, Brazil and China have considerably lower yields of maize relative to the United States. However, South Africa is the largest producer and has highest grain yield/ha in Africa.

Low grain yields in South Africa could be attributed by poor genetic improvements and poor management practices. There are few heterotic patterns that can exploit heterosis which are established in South Africa hence this could also lead to low yields compared to countries like United States of America where there is a wide range of heterotic patterns.

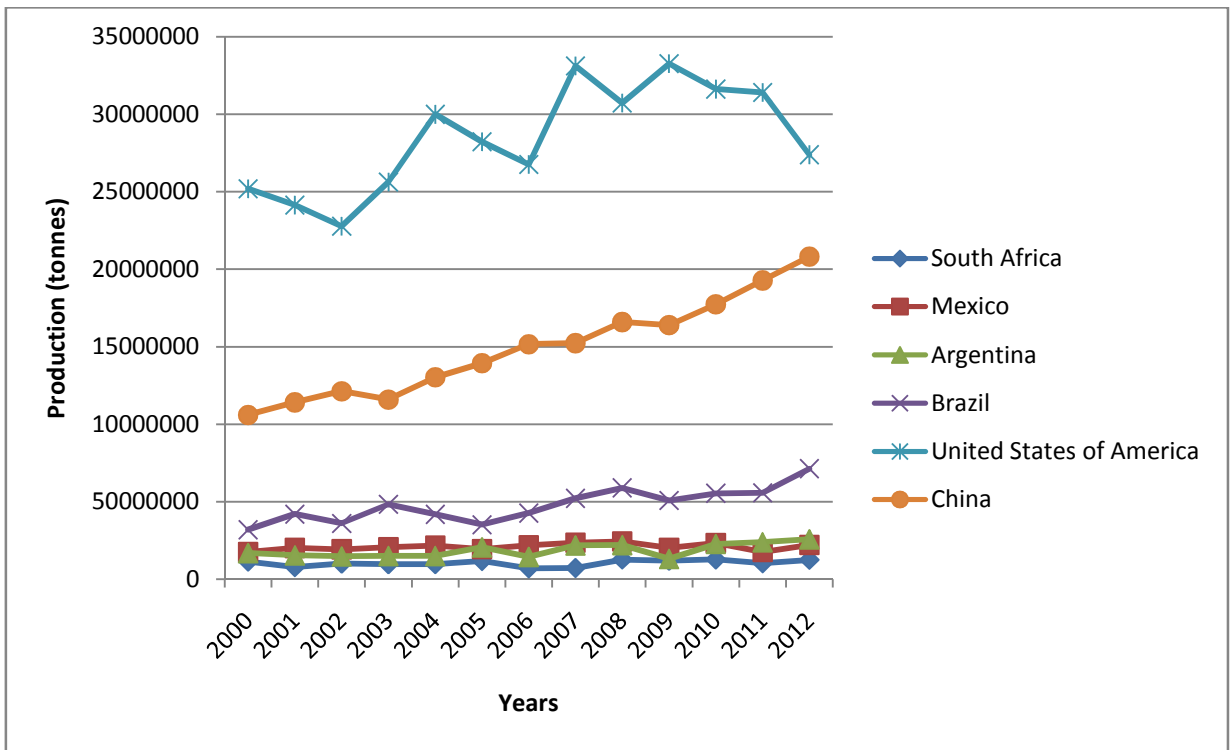


Figure 1.1: Global maize production (FAOSTAT, 2013).

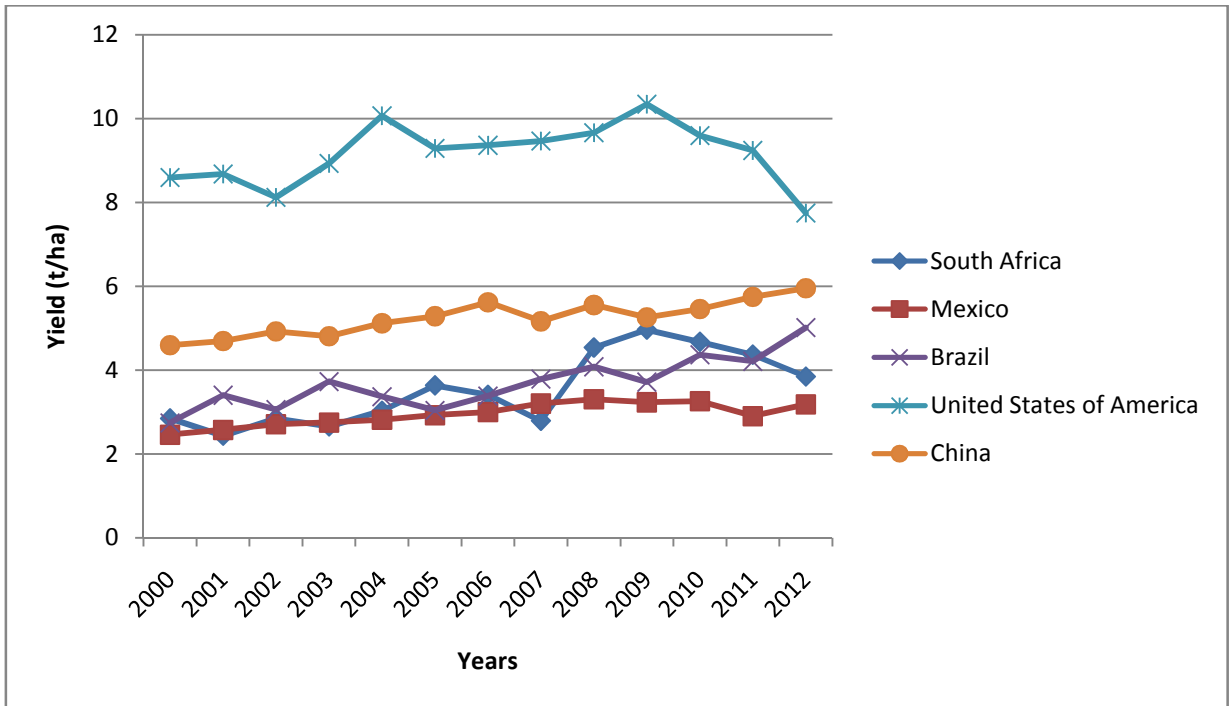


Figure 1.2: Global grain yield (t/ha) of maize (FAOSTAT, 2013).

Maize yields in South Africa have been fluctuating since the year 2000. In the past four years (2009 to 2012) there has been a gradual decline in the yields of maize (Figure 1.3). This was mainly due to drought stress associated with climate changes experienced in the country since most maize is grown under rain fed conditions (Department of Agriculture, 2012). A shift in climate can delay the onset of rainfall, cause prolonged dry spells which will affect the performance of crops leading to low yields. Some of the main maize producing areas in South Africa may have received below average rainfall leading to decline in yields (Department of Agriculture, 2012) (Figure 1.3).

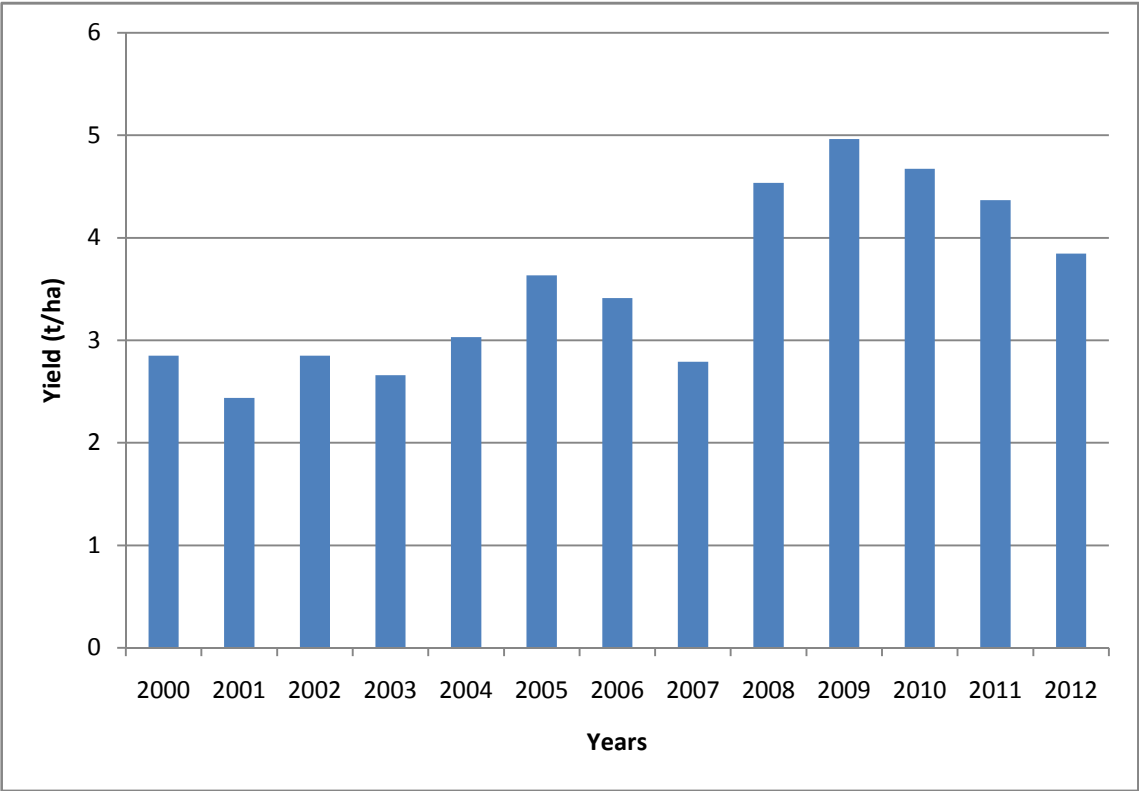


Figure 1.3: Maize production in South Africa from 2008 to 2012 (FAOSTAT, 2013).

Significance of popcorn

Popcorn is a popular snack worldwide and is becoming widely accepted in many parts of Africa including South Africa. Due to lack of a wide range of suitable varieties of popcorn in South Africa there is no record of its total production. The cause for the discrepancy in production is mainly due to slow progress in breeding superior popcorn open pollinated varieties, hybrids and poor agronomic traits. The fact that popcorn is regarded as snack food has resulted in little resources being channeled towards the production of hybrids compared to that of dent and flint maize, which are regarded as food security crops. Hence, South Africa has been importing popcorn which is not adapted to local conditions. In South Africa, popcorn breeding efforts were last reported in 1954 (Josephson, 1954).

The role of hybrids in maize production

Yield increase has been the major aim in many plant breeding programs. Hybrids can be developed from single, double and three-way crosses. Single crosses have been reported to be the best sources of germplasm because they are high yielding and are adapted to a wide range of environments compared to three-way and double-cross hybrids (Boonlertnirun et al., 2012). Therefore in the current study single cross hybrids that exhibit maximum heterosis were pursued.

Knowledge of genetic diversity in maize is important in designing hybrids. Genetic distance provides useful information on divergence of different genotypes that exploit heterosis (Azad et al., 2012). Most reports on the relationship between genetic distance and heterosis are that yield increases with increasing divergence of parental lines. Therefore, it is very crucial to know the divergence between lines so that different heterotic groups can be assigned to make crosses from distantly related genotypes in order to increase yield potential. Studies on the genetic distances between inbred lines of both popcorn and normal maize (flint and dent) are few amidst controversy regarding the relationship between hybrid performance and genetic distance. It is, therefore, prudent to investigate whether or not genetic distance can be used to predict hybrid performance.

Problem Statement

Currently there is a yield plateau in maize due to limited availability of improved varieties and production technologies for farmers. Grain yield is generally low yet food requirements have increased tremendously leading to a huge gap between population growth and food production in SSA. This is mainly attributed to poor genetic improvements in maize and equally poor management practices. There are no new and effective hybrid patterns that have been developed especially for stress prone environments. This applies to both popcorn and normal maize hybrids.

There is not even a single popcorn hybrid that has been developed in South Africa since 1954; hence farmers rely on imported seed from North America which is not adapted to the local conditions. This prevents adoption of popcorn by small-scale farmers in marginal environments where the crop would be grown with minimum resources such as inadequate fertiliser, water and crop protection.

Knowledge of genetic distances between maize germplasm lines can be used to design new hybrids. Unfortunately, genetic distances among breeding germplasm lines that have been developed in the maize programme at the University of KwaZulu-Natal has not been established. Importantly, the relationship between genetic distance and heterosis has not been identified yet knowledge of this relationship is important for designing new heterotic patterns. There is need also to quantify diversity of hybrids in South Africa. The information that will be generated can be used to devise an appropriate breeding strategy that aims to develop hybrids with adaptation ability under the production conditions of South Africa.

Research objectives and hypotheses

The goal of this study was to identify new heterotic patterns and diversity among dent, flint and popcorn maize hybrids in order to improve grain yield.

Specific objectives

The following specific objectives were pursued in the study:

1. To determine standard heterosis, the levels of variation and heritability for phenotypic traits in dent and flint maize hybrids
2. To determine the association between genetic distances and phenotypic traits in dent and flint maize hybrids
3. To determine the levels of mid-parent heterosis in popcorn hybrids
4. To determine the effect of secondary traits on grain yield in dent, flint and popcorn hybrids
5. To determine the level of genetic diversity and the relationship between traits in widely grown selected hybrids in Southern Africa
6. To compare the effectiveness of phenotypic analysis models for determining genetic distances between hybrids.

Research questions

The study aimed to answer the following research questions:

1. What are the levels of standard heterosis, variation and heritability for phenotypic traits in dent and flint maize hybrids?
2. What is the level of association between genetic distances and phenotypic traits in dent and flint maize hybrids?
3. What is the level of mid-parent heterosis in popcorn hybrids?
4. What is the effect of secondary traits on grain yield in dent, flint and popcorn hybrids?
5. What is the level of genetic diversity and the relationship between traits in widely grown selected hybrids in Southern Africa?
6. What is the effect of phenotypic analysis models in determining genetic distances between hybrids?

Research Hypotheses

The following hypotheses were tested:

1. There is a significant difference in the levels of standard heterosis, variation and heritability for phenotypic traits in dent and flint maize hybrids
2. There is a significant association between genetic distances and phenotypic traits in dent and flint maize hybrids
3. There is a significant difference in the levels of mid-parent heterosis in popcorn hybrids
4. Secondary traits have significant direct effects on grain yield which can be exploited to enhance yield in dent, flint and popcorn hybrids
5. There is significant genetic diversity and the relationships between traits in widely grown selected hybrids in Southern Africa
6. The effectiveness of phenotypic analysis models for determining genetic distances between hybrids increases when many traits are included in the model.

Outline of the dissertation

This dissertation is made up of five chapters as shown below:

Chapter 1: Literature Review

Chapter 2: Heterosis, heritability, genetic distance and path coefficient analysis of yield and related traits in dent and flint maize hybrids

Chapter 3: Heterosis and path coefficient analysis in popcorn experimental hybrids

Chapter 4: Diversity and path coefficient analysis of Southern African maize hybrids

Chapter 5: General discussion, conclusions and future directions

All, except chapter 1 are written in IMRD format that include Introduction, Materials and Methods, Results and Discussion.

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CHAPTER ONE

LITERATURE REVIEW

1.1 Introduction

This chapter reviews the present body of knowledge for the research context and discusses the importance of maize and types of maize. It also reviews heterosis and its relationship with genetic distance in dent and flint hybrids, heritability, correlation and path analysis in dent and flint hybrids, heterosis and path analysis in popcorn hybrids, genetic diversity among Southern African hybrids based on markers and phenotypic data. Conclusions drawn from this review are provided at the end of the chapter.

1.2 Global Importance of maize

Maize (*Zea mays* L.) is one of the most important food security crops in developing countries (Cheres et al., 2000; Mohan et al., 2011; Avinashe et al., 2012). Maize is extensively grown from sea level to elevations exceeding 3000m throughout the world in many ecological environments (M'mboyi et al., 2010). More than 300 million Africans depend on maize as their main food source (M'mboyi et al., 2010). Maize is considered as one of the most important cereal crops for human food and livestock feed (Cheres et al., 2000; Shete et al., 2011; Jawaharlal et al., 2012; Sumalini, 2012). In SSA maize is used directly for human food with more than 100kg consumption per capita (Adeyemo et al., 2011). The demand for maize will increase to 852 million MT by 2020 (FAOSTAT, 2010), but its yield potential has remained low in SSA (Badu-Apraku and Oyekunle, 2012) with implications for food security. The yield of maize fails to match the demand due to biotic and abiotic stress such as diseases, pests and weeds, drought, low soil fertility and soil acidity (M'mboyi et al., 2010).

1.3 Types of maize

There is diversity in maize which is reflected through grain texture such as dent or flint (Anderson et al., 1952). The dent and flint maize are generally used for grain or food security especially in SSA. However, as society improves there will be interest in speciality maize such as that which is high in quality protein, food corn, waxy maize, high amylase maize, sweet and popping maize. However, there is a shortage of speciality maize hybrids in SSA. Therefore, research was conducted to develop strategies for breeding dent, flint and popcorn hybrids.

1.3.1 Popcorn

Popcorn (*Zea mays* Everta.) is a popular snack food in the United States and is becoming accepted in many other parts of the world including South Africa (Dhliwayo, 2008). Regardless of its importance, the genetic improvement for popcorn production is being hindered by yield, stalk strength, insect pest resistance and disease resistance as these have not been given much attention compared to dent and flint maize (Ziegler, 2000). For popcorn to be accepted in the industry, it must meet distinctive and very strict quality standards (Ziegler, 2000).

One of main characteristics of popcorn which makes it different from all other types of maize is the ability to pop and produce flakes when heat is applied (Dhliwayo, 2008). Grain yield is the most important parameters in popcorn (Zorica et al., 2008). Procedures and techniques used in popcorn breeding program are almost similar to those used for dent corn with some minor adjustments needed (Erazo-Barradas, 2009). Generally popcorn has a narrow genetic base (Miranda et al., 2008; Zorica et al., 2008; Vijayabharathi et al., 2009). This is detrimental for popcorn breeding because a certain level of parental divergence is needed to create productive hybrids. One of the most essential traits in popcorn, as well as all other types of corn is yield which is mainly affected by the genotype (Zorica et al., 2008; Vijayabharathi et al., 2009). However, there are no enough improvements that have been made in popcorn hybrids especially for tropical environments.

Developing countries are lagging behind in popcorn breeding and production due to challenges of inferior germplasm and poor agronomic traits of popcorn varieties. Furthermore, it is also attributed to less effort that has been rendered towards the improvement of popcorn breeding (Ziegler, 1983). Limited breeding programs in Africa are a major contributing factor limiting popcorn production. Most imported varieties are not adapted to stress-prone environments, and cannot be produced locally. For that reason, plant breeders should aim to develop well-adapted hybrids with high yield potential (Zorica et al., 2008; Öz and Kapar, 2011).

1.3.2 Flint corn

Flint corn (*Zea mays Indurata*.) also known as Indian corn is used mainly for human consumption and livestock feed. Flint corn is characterized by a hard outer shell and the kernel colour range from white to red. Flint corn tends to mature earlier compared to dent corn and it has a superior germination capacity especially under cold conditions (Dowswell et al., 1996). Under ideal conditions flint corn can pop, however the kernels are harder compared to popcorn (Smith, 1999). Doebley et al. (1986) reported that Northern Flint corn of the Eastern North America has contributed effectively to most of the germplasm found in today's corn because it is widely spread, it yields well and it is also adapted to many conditions. Soengas et al. (2003) reported that in the commercial industry the most commonly used heterotic pattern to produce flint hybrids is the Reid x Lancaster. However, in Europe some patterns such as European flint x Corn Belt dent have also been widely used. These combinations have allowed expansion of maize all over the world. Some studies have reported high heterosis between Yugoslavia and Corn Belt germplasm and between Spanish and Corn Belt populations (Soengas et al., 2003). The presence of heterotic patterns indicated that there is heterosis within maize population (Soengas et al., 2003). Boppenmaier et al. (1993) reported high heterosis in flint x flint compared to flint x dent and dent x dent crosses.

1.3.3 Dent corn

Dent corn (*Zea mays Indenata*) also known as field corn is mainly used as a food security commodity in most parts of the world especially in developing countries. Dent corn is also used for livestock feed in both developed and developing countries. Dent corn is either white or yellow in colour. It contains both hard and soft starch that becomes indented at maturity. Dent corn is also characterised by the presence of a horny endosperm at the back of the kernel (Logsdon, 2009). Unlike popcorn or flint corn, dent corn does not pop even under ideal conditions for popping. The Reid Yellow dent group has been reported to be the largest group which has made significant contributions to commercial hybrids together with North American dent (Mikel and Dudley, 2006). Boppenmaier et al. (1992) reported that higher yields were obtained when dent x dent were crossed than dent x flint. Dent corn has been reported to be the best in the development of hybrids to be grown in mild summer environments (Boppenmaier et al., 1992).

1.4 Heterosis

Fehr. (1991) defined heterosis as the superiority in performance of hybrid individuals compared with their parents. This implies that the hybrids obtained have more vigour than their parents. Falconer and Mackay. (1996) defined heterosis or hybrid vigour as the difference between the hybrid and the mean of the two parents. This difference is called mid-parent heterosis. The type of parents chosen and measurement of trait determine the level of heterosis in maize that is why it is important to select superior parents (Ali et al., 2012). Better parent heterosis quantifies the performance of the F1 hybrid over the better performing parent (Springer and Stupar, 2007). Heterosis has been used in breeding and production of many crops and in maize an estimate of 15% per annum on yield increase has been reported through the use of hybrids (Iqbal et al., 2010). The significance of heterosis therefore implies that hybrid varieties can be developed to augment grain yield (Fato, 2010).

Literature indicates three theories behind heterosis (Crow, 1948; Mayo, 1987). Heterosis under the dominance hypothesis is produced by masking of deleterious recessive alleles in one strain by dominant or partially dominant alleles in the second strain (Hallauer and

Miranda, 1988; Iqbal et al., 2010). While on the other hand, under the over-dominance hypothesis heterosis is due to the heterozygosity superiority, and therefore, increased vigour is proportional to the amount of heterozygosity (Crow, 1948; Iqbal et al., 2010; Ali et al., 2012). Lastly, epistasis is mainly based on the basis of interactions among genes at different loci (Cockerham and Zeng, 1996; Iqbal et al., 2010).

Heterosis is usually measured against standard control, better-parent or mid-parent heterosis. Positive standard heterosis for grain yield was reported by Kustanto et al. (2012) ranging from 1.7% to 212.4%. Similar results were also reported by Gurung et al. (2013) although on a small magnitude where standard heterosis for grain yield ranged from 39.4% to 47.8%. Furthermore, Amiruzzaman et al. (2010) reported that standard heterosis ranged from 7.60% to 39.71% for grain yield. Considerable amount of heterosis were reported by Saidaiah et al. (2008) where out of 40 hybrids, the heterosis for grain yield per plant was significant in 23 crosses over better-parent and 11 crosses over standard check. Hiremath et al. (2013) reported a range of -31.58% to 22.11% over the standard check on grain yield. However, Avinashe et al. (2013) reported that among 45 crosses, only three crosses recorded maximum significant and positive standard heterosis for grain yield per plant.

1.5 Heterotic grouping and heterotic patterns in maize

Heterotic group refers to “a group of related or unrelated genotypes from the same or different populations which display similar combining ability and heterotic response when crossed with genotypes from other genetically distinct germplasm groups (Melchinger and Gumber, 1998). Heterotic patterns refer to “a specific pair of inbred lines or varieties which express high heterosis in crosses” (Lu and Xu, 2010). Genetically different lines have high hybrid vigour compared to genetically similar lines which express inbreeding depression. Therefore, preferred lines are those that have different genetic backgrounds and are in different heterotic groups (Fato, 2010). This can be achieved by studying genetic distance between the lines before crosses are made. Classification of inbred lines into heterotic groups facilitates the exploitation of heterosis in maize, which can contribute to hybrid performance (Bidhendi et al., 2011). Therefore, knowledge of genetic diversity of maize germplasm is important for designing a hybrid maize breeding program (Hallauer and Miranda, 1988).

1.6 Methods used in heterotic grouping

One of the foundation pillars of maize breeding is the establishment of heterotic groups. Although several methods have been employed in the heterotic grouping of maize lines, fundamental methodological procedures are not yet fully understood (Fato, 2010). Currently, a number of methods have been established and are being used to assign maize lines into heterotic groups and these include clustering by phenotypic traits and molecular markers (Lu and Xu, 2010). The method of clustering by molecular markers is the most commonly used because it has high resolution power though at times it is constrained by the kind and quantity of primers used (Lu and Xu, 2010). A number of molecular markers such as Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR) and Single Nucleotide Polymorphism (SNP) makers have been used to demonstrate the efficiency of the identification of maize heterotic groups (Aguiar et al., 2008). These markers arise from different classes of DNA mutations such as substitution (point mutation), rearrangements (insertion or deletion) or errors in replication of DNA.

Molecular markers have advantages over other methods, because they show genetic differences on a more detailed level, they are not affected by the environment and they involve methods that provide fast results in determining genetic diversity (Leal et al., 2010). A lot of research work has been done in maize in which markers were used to identify heterotic groups of maize lines (Aguiar et al., 2008; Dandolin et al., 2008). Nowadays a reliable cost effective marker that allows better characterization of genetic resources and breeding materials is of wide choice. SNP markers have become the ideal marker for genetic research because they are abundant and are evenly distributed throughout the genome (Semagn et al., 2012). They are biallelic, codominant and have lower information content compared to SSR markers and they are also amenable to high throughput methods (Semagn et al., 2012). Although RFLP and SSR markers possess most of these attributes they are expensive to genotype hence there is a shift towards SNP as a marker of choice because they are cheaper (Yan et al., 2010). Therefore, in this study SNP markers were used.

The use of phenotypic traits in clustering inbred lines into heterotic groups is also being used by breeders. Phenotypic traits are more relevant in the field and they are a true ideotype (Varshrey et al., 2005). Although clustering using phenotypic traits has a number of disadvantages, the method cannot be completely discarded because there are some breeders who still prefer using phenotypic traits to cluster inbred lines into heterotic groups (Cheres et al., 2000).

Currently, there are nine main heterotic groups (SC, N3, K, P, I, M, F, CIMMTY-A, CYMMYT-B) of elite inbred lines that are used for maize breeding program in Eastern and Southern Africa (Gevers and Whyte, 1987; Olver, 1998; CIMMYT, 2001; Mickelson et al., 2001). Therefore, there is need to develop new heterotic patterns in order to increase yield potential.

1.7 Genetic Distance

Genetic distance in maize plays a very important role in maize breeding programs as it provides useful information of divergent genotypes that exploit heterosis (Dandolin et al., 2008; Leal et al., 2010). Both molecular markers and phenotypic traits can be used to determine genetic distance (GD) among groups of maize genotypes in order to identify new heterotic patterns (Legesse et al., 2008). Information on GD between genotypes has been broadly used in assigning maize lines into different heterotic groups (Cheres et al., 2000). Berta et al. (2007) reported that the main technique to measure GD is based on the use of phenotypic characteristics. This is in contrast with Cheres et al. (2000) who reported that GD can also be estimated without phenotyping because the use of phenotypic traits is time consuming, laborious and it is likely to give less accurate results. Molecular markers are a powerful tool for defining heterotic groups and assigning inbred lines into existing heterotic group (Pajic and Srdic, 2007). The Genetic distance between inbred lines obtained through the use of molecular markers in plant breeding is being considered a viable, alternative for predictions of hybrid performance and heterosis in crosses (Aguiar et al., 2008).

Various statistical methods are used to estimate genetic distance in maize breeding programs depending on the data set. The commonly used methods include the Nei and Li coefficient, Jaccards' coefficient, Simple matching coefficient, Modified Roger' distance, Mahalanobis

(D^2) and Euclidean distance (Darbeshwar, 2000; Mohammadi and Prasanna, 2003). The Mahalanobis requires data of more than one replication to estimate distance hence its use is limited (Bertan et al., 2007). Many breeders use hierarchical methods to group genotypes by a process that repeats itself at many levels forming a dendrogram without concern for the number of groups formed (Bertan et al., 2007). Different cluster methods can then be used depending on the procedure that is most suitable for the data-set. Euclidean distance discriminates genotypes that are very close to each other; hence it was used in this study.

1.8 Heterosis and its relationship with Genetic Distance in dent and flint hybrids

Genetic distance has been broadly associated with heterosis in maize using either molecular markers or phenotypic data (George et al., 2011). The relationship between heterosis and GD in maize showed that yield increases with increasing divergence of parental lines (Moll et al., 1965; Phumichai et al., 2008; Boonlertnirun et al., 2012). However, Moll et al. (1965) reported that this relationship will not necessarily hold throughout the whole range of diversity in the species. This is in support with previous results from George et al. (2011) where no correlation was observed between GD and heterosis when GD was (>0.77). Therefore, this poses a challenge to maize breeders intending to identify inbred lines that can exploit heterosis because it cannot be guaranteed that inbred lines from different heterotic groups will always have high hybrid vigour for yield. Wegary et al. (2013) reported that the magnitude and degree of heterosis depends on the relative performance of the inbred parents and the hybrids. The environment can also cause different effects on the performance of hybrids thereby altering the relationship between GD and heterosis (Wegary et al., 2013).

Subramanian and Subbaraman (2012) studied genetic diversity and heterosis among 38 maize inbred lines using RAPD markers. They found that RAPD markers were effective in determining genetic diversity among the inbred lines by clustering them into different heterotic groups. In addition, genetic divergence in lines was significantly correlated to hybrid yield. In contrast studies conducted by George et al. (2011) found no correlation between GD and F1 grain yield, mid-parent heterosis and high-parent heterosis when GD was >0.77 . This implies that when parents become extremely diverse the relationship between heterosis and GD is no longer observed. Semagn et al. (2012) did a study to investigate the usefulness of SNP markers in identifying heterotic groups. They found out that SNP markers could not

show a clear separation of heterotic groups A and B. This was because many of the inbred lines used in the study were developed from the same genetic pool.

Genetic distance among inbred lines is also estimated using phenotypic data such as the pedigree record of inbred lines (Cheres et al., 2000; Enoki et al., 2002). However, phenotypic data has a number of disadvantages as they are affected by the environment (Cheres et al., 2000; Enoki et al., 2002). Therefore, the use of phenotypic traits in determining genetic diversity can be boosted by the use of molecular markers. This is supported by Karanja et al. (2010) who did studies on the correlation between molecular and morphological traits in maize inbred lines. They found out that both morphological data and SSR markers managed to cluster the maize inbred lines into different heterotic groups. Furthermore, Shahrokhi and Khavarikhorasani (2013) reported on the effectiveness of pedigree data as it grouped 28 commercial corn hybrids into 4 main heterotic groups.

1.9 Heterosis in popcorn

Popcorn has a narrow genetic base (Dandolin et al., 2008; Miranda et al., 2008; Leal et al., 2010). This poses challenges in popcorn breeding because a certain level of parental divergence is required for creation of productive hybrids (Dandolin et al., 2008). Popcorn breeding is very scarce in many parts of Africa including South Africa because there are limited numbers of genetically improved varieties. Saavedra et al. (2013) suggested that the genetic base of breeding populations of popcorn could be enhanced through introduction of new germplasm. Furthermore, Melani and Carena (2005) indicated that the success of a popcorn breeding program depends on parental selection and identification of heterotic groups. In several studies conducted on popcorn, parent selection and the type of crossing are seen to be very important in breeding programs in order to develop superior hybrids. Therefore, knowledge of the genetic diversity on the available germplasm is very useful for optimal design of breeding program in popcorn improvement (Li et al., 2004).

1.10 Genetic Diversity among South African hybrids based on markers and phenotypic data

Genetic diversity and the levels of genetic variations in maize can be estimated using both molecular markers and phenotypic traits (Karanja et al., 2010). No marker can exclusively give all the information needed in plant breeding, without support from other techniques. This is more likely in maize as it exhibits a wider range of morphological and molecular dynamism than many other crops (Karanja et al., 2010). The increasing demand for maize in developing countries due to population growth requires the application of crop improvement tools to increase yields (Dhliwayo et al., 2009). In South Africa only a few heterotic groups have been identified which are currently used in the local breeding programs.

The use of phenotypic traits in diversity studies has not been able to reflect the genetic constitution in maize because it is influenced by the environment (Sharma et al., 2010). However, it can never be excluded in crop improvement as it can be boosted by the use of molecular markers (Karanja et al., 2010). In order to widen the genetic base for commercial hybrids there is need to seek for more diversity among inbred lines (Karanja et al., 2010). Wen et al. (2011) elaborated the need to study diversity using both pedigree data and molecular markers because some markers have been reported to have some discrepancies as groups of genetically similar germplasm based on pedigree information could not be confirmed using markers.

Schaefer and Bernardo (2013) obtained five clusters from 284 maize inbred lines using SNP markers. Similar results were also reported by Yang et al. (2011) where the same number of clusters was observed by using SNP and SSR markers. Losa et al. (2011) reported that AFLP markers were effective in assigning maize inbred lines into heterotic groups for superior line development with the aim of maximizing heterosis and yield performance. Pedigree analysis placed 119 inbred maize lines into eight groups and SSR markers resulted in clustering the inbred lines into 10 groups (Reid et al., 2011). The use of pedigree information to cluster maize inbred lines was also reported by Wen et al. (2011) where they elucidated that lines related by pedigree tend to cluster together and nine main subsets of lines were determined based on pedigree information.

1.11 Heritability in maize

Heritability was defined by Mayo (1987) as the proportion of the total variance of a character that is attributable to genetic factors as opposed to environmental factors. Fehr (1991) highlighted that heritability shows the relative importance of genetic and non-genetic factors in the expression of phenotypic differences among genotypes in a population. Heritability can be expressed into two senses, the narrow sense and the broad sense. Narrow sense heritability is the ratio of additive genetic variance to the phenotypic variance (Fehr, 1991). Broad sense heritability is the proportion of the total genotypic variance to the phenotypic variance which include additive, dominance and epistatic variance (Fehr, 1991; Falconer and Mackay, 1996). Heritability can be estimated by using either variance components or parent-offspring regression (Fehr, 1991). Smalley et al. (2004) reported that heritability relies on the assumption that more closely related individuals resemble one another than those individuals that are less closely related.

Heritability values differ among populations and they vary for different traits. Wannows et al. (2010) reported high heritability estimates of 85% plant height, 83% for ear height, 82% for physiological maturity, 73% for ear length, and moderate heritability estimates of 34% for silking dates and 39% for grain yield. In studies conducted by Smalley et al. (2004) they reported low heritability estimates of 7% for grain yield, moderate heritability estimates of 51% ear height and 56% for plant height, and lastly high heritability estimates of 61% for number of tassel branches. Saleh et al. (2002) conducted a study on heritability estimates on tropical maize on grain yield. Low to moderate heritability was reported for plant height and ear height. Low heritability estimates are an indication that environmental factors have played a major role compared to genetic factors. Hallauer and Miranda (1988) reported moderate heritability of 41% for grain yield and high heritability of 66% and 81% for ear height and plant height respectively. Highest heritability estimates were found for days to 50% flowering (79.1%) and plant height (36.4%) as reported by Idris and Mohammed (2012). Scion et al. (2010) detected a high narrow sense heritability value of 88% on grain yield. Similarly, Rafiq et al. (2010) found high narrow sense heritability of 91% on grain yield.

1.12 Genotype by Environment Interaction in maize

Genotype x Environmental Interaction (GEI) is when the relative performance of genotypes changes from one environment to another (Crossa et al., 1999; Reymond et al., 2004). Yang and Baker (1991) reported that inconsistent differences in the performance of genotypes in different environments could be due to the differences in response of the same set of genes to different environments or the expression of different sets of genes in different environments. Specifically, some studies have suggested that GEI could be due to inconsistency of genotypes in response to factors like temperature, soil type, fertility level soil moisture or pest and diseases from location to location and year to year.

A significant GEI causes a reduction in breeding programs especially for main traits such as grain yield. For this reason breeders have invested a lot of time and resources trying to investigate the implications of the GEI in plant breeding (Dehghani et al., 2009). GEI is a challenge to plant breeders because it complicates breeding procedures and limit the usefulness of selection in any individual environment (Kang and Gorman, 1989; Crossa et al., 1999). Therefore, knowledge of the presence of GEI can help breeders to make informed decisions in order to optimise the breeding methods, selection intensity and testing procedures (Issa, 2009).

1.13 Correlation analysis

Mohanani (2010) defined correlation as the association of characters that exhibit some trends of change. The correlation can either be positive or negative (Mohanani, 2010). A correlation coefficient can help the breeder to ascertain the magnitude of association of secondary traits with yield (Yousuf and Saleem, 2001). It measures the mutual association between a pair of traits when more than two traits are involved, though they may not give a clear picture of the importance of each trait in determining grain yield (Sre kov et al., 2010). A significant association suggests that the traits can be used as primary selection for grain yield (Ojo et al., 2006). Bolanos and Edmeades (1996) reported that the interpretation of the correlation between yield and its secondary traits should be done with caution as results are generally affected by the presence of outliers.

Sreckov et al. (2011) reported that grain yield was significantly and positively correlated with kernel row number, ear length and 100-kernel weight. Ojo et al. (2006) found that shelling percentage, ear weight, days from planting to silking and plant height exhibited positive correlation coefficients with grain yield per plant. Association studies indicated that traits such as plant height, ear height, ear length, and number of grains per row, number of grains per ear and grain weight showed significant and positive association with grain yield (Selvaraj and Nagarajan, 2011). Similar results were observed by Hefny (2011) who found that ear diameter, ear weight, number of rows per ear and number of grains per row exhibited positive significant correlations with grain yield. Days to tasseling and days to silking showed positive non-significant association with grain yield (Selvaraj and Nagarajan, 2011).

Hefny (2011) reported that 50% tasseling and silking were highly correlated with each other and they associated negatively with ear diameter, grains per row and 100 grain weight. Singh et al. (2006) reported significantly positive correlations between grain yield, plant height, ear height and number of ears. Majid and Roza (2010) found that grain yield was correlated with ear length, ear diameter, and plant height. It had been observed that grain yield was significant and correlated with ear length, rows per ear, plant height and ear prolificacy (Moradi and Azarpour, 2011). Golbashy et al. (2011) observed that plant height was significantly correlated with ear height.

1.14 Path Analysis

Grain yield is the most important trait in maize breeding programs. It is the most complex quantitative trait that is determined by several yield components (Kumar et al., 2011). The contribution of each trait to grain yield potential is essential in maize hybrids. Therefore, knowledge on direction and magnitude of association between yield and its components is important for plant breeding (Kumar et al., 2011). Path coefficient analysis is broadly used in plant breeding programs to determine the nature of relationships between grain yield and its contributing components (Zarei et al., 2012). It identifies those components with significant direct effects on yield for potential use as selection criteria (Zarei et al., 2012). Sreckov et al. (2011) highlighted that path analysis also provides information on indirect effects on yield via

other yield components. Path coefficient analysis equips the breeder with knowledge on the traits that determine yield so that they can make decisions on whether to practice direct or indirect selection (Makanda et al., 2009).

Rafiq et al. (2010) reported that the highest direct effects on grain yield were exhibited by grain weight, kernel row per ear and ear length. Tassel number exhibited large and positive direct effects on grain yield while days from planting to silking had highest negative direct effects on grain yield followed by plant height (Ojo et al., 2006). Studies conducted by Jayakumar et al. (2007), showed that grains per row, ear length, days to tasseling and plant height exhibited positive direct effects on grain yield. Kernel rows, days to silking, grain weight, days to maturity and shelling percentage recorded high values for negative direct effects on grain yield (Jayakumar et al., 2007). Akbar (2008) found a positive direct influence of ear height on grain yield. Kernel width, number of kernels per ear, number of kernels per row and cob diameter had positive direct effects on grain yield (Ali et al., 2012). In another study by Sofi and Rather (2007) path analysis indicated that 100 grain weight had the highest positive direct effect on grain yield followed by kernels per row, number of kernel rows per ear and ear length.

1.15 The relationship between yield and secondary traits

Secondary traits are very useful in selection for improved maize yields. A desirable secondary trait must be genetically associated with final grain yield, have a level of heritability and genetically diverse within species (Bertan et al., 2007; Lu et al., 2011a). Bertan et al. (2007) reported that secondary traits have genetic variance and heritability that increase under difficult conditions and they are useful to improve efficiency when grain yield is reduced.

Knowledge on the genetic association between traits is very important for the establishment of selection criteria (Nastasi et al., 2010). This is because improving one trait might concurrently change the expression of another trait due to their relationships. Maize grain yield is a quantitative trait in nature and it is controlled by many genes. Therefore, effective yield improvement and simultaneous improvement in yield components are very important (Bello and Olaoye, 2010).

Selvaraj and Nagarajan (2011) found that plant height, ear height and ear length and grain weight showed significant positive association with grain yield. On the other hand, days to tasseling and days to silking showed positive non-significant association with grain yield. The results were in agreement with Bello and Olaoye (2010) who reported positive and significant correlation between days to 50% tasseling and grain yield. However, these results were in contrast with Al-Yemeni (2011) who found negative association between days to 50% silking and days to maturity. The discrepancy between the results is mainly due to differences in germplasm used and the environment. Hence there is need to evaluate genotypes under different environments to determine the effects of genotype x environment on the correlation between yields and secondary traits.

1.16 Conclusion

Agriculture in the 21st century faces multiple challenges; it has to produce food to feed the growing global population. This can only be achieved if different crop improvement strategies are integrated to improve the yield of maize in SSA. Yield is the most important parameter in maize and many factors that influence yield in maize have to be considered. Good genetic variation between hybrids is essential for desirable breeding program. For successful development of superior hybrids, associations among adaptive traits, genetic diversity and heterotic patterns need to be fully understood. Generally hybrids from different heterotic groups are more likely to exhibit heterosis. There are only nine heterotic groups that are being used for maize breeding programs in Eastern and Southern Africa, therefore it is important to establish more heterotic groups and boost the yield of maize in South Africa. Although studies have been done on correlation and path analysis there is very little information that has been reported on the significant direct effects on yield.

The use of molecular markers is a powerful tool in the study of genetic diversity which plays a very crucial role for future breeding progress. Phenotypic traits can also be used to discriminate hybrids into clusters though they do not always reflect the genetic constitution in maize because of environmental influences but they can never be excluded in crop improvement as they can be boosted by the use of molecular markers. A very few papers

have reported on the use of both molecular markers and phenotypic traits in discriminating hybrids into clusters, hence there is need to evaluate these methods and see their effectiveness. Therefore, molecular tools have been effectively integrated with phenotypic traits in this current study. There is no literature regarding the levels of diversity in South Africa which has implications on the maize breeding because of climate changes and other environmental challenges, therefore, this call for the need to study the diversity of widely grown hybrids in South Africa.

There is not even a single popcorn hybrid that has been developed in South Africa since 1954 hence farmers rely on imported seed from North America which is not adapted to the local conditions. This prevents adoption of popcorn by small-scale farmers in marginal environments where the crop is grown with minimal resources such as inadequate fertiliser, water and crop protection. Hence there is need to breed for popcorn germplasm that is adapted to SSA. There is insufficient research that has been done on heterosis in popcorn. Similar amounts of resources have not been put towards popcorn breeding compared to dent and flint maize because it is regarded as a snack food. However, there is an increase in the demand for popcorn in developing countries forcing breeders to establish popcorn hybrids that are adapted to local conditions.

1.17 References

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CHAPTER TWO

Heterosis, heritability, genetic distance and path coefficient analysis of yield and related traits in dent and flint maize hybrids

Abstract

Yield and yield components can be greatly enhanced by exploiting heterosis in maize hybrids. However, breeders require crucial genetic information regarding the maize inbred lines to design hybrids. The relationship between genetic distance and heterosis in maize hybrids would make it easy to select superior hybrids. The objectives of the study were to determine heterosis, association between genetic distance and heterosis and the levels of genetic variation, association between secondary traits and grain yield and heritability for phenotypic traits in dent and flint maize hybrids. Therefore, 190 normal maize hybrids were evaluated at two sites. The experiments were laid out as augmented 10 x 19 alpha lattice designs. The data was analysed using mixed models in SAS. Significant genetic variation and new heterotic patterns were observed. The results also indicated that the association between genetic distance and yield potential in maize hybrids depended on the environment. There was a positive and significant correlation between yield and genetic distance in one environment ($r=0.31$; $P<0.01$); but there was no significant relationship in the other environment. Grain yield was highly heritable at both sites indicating that direct selection of hybrids for grain yield potential would be very effective. Considerable amounts of heterosis were observed at both sites, thus experimental hybrids which performed better than the standard controls would be advanced. The main direct factors contributing to yield potential were ear prolificacy, plant height, ear height, ear length and ear diameter indicating that direct selection for these traits would be effective to improve grain yield of maize hybrids. Therefore these traits can be used as the primary selection criteria for grain yield. However, indirect effects of plant height, plant aspect, ear height, and grain texture via ear height and ear position could also be exploited to improve yield potential. Consistent with the literature, new heterotic patterns were observed between and within heterotic groups of maize. With respect to grain texture, productive patterns such as flint x flint, dent x flint and dent x dent were also observed. The two inbred lines (DXL124 and DXL158) that were involved in most of the top 10 hybrids will be adopted as new potential testers for specific combining ability in future studies.

Key words: maize, heterosis, genetic distance, yield components, heritability, genetic variation

2 Introduction

Yield and yield components can be greatly enhanced by exploiting heterosis in single cross maize hybrids. Maize is one of the most important crops grown in the world (Pavan et al., 2011a; Avinashe et al., 2012). However, the yield plateau in maize has never been broken because of challenges that breeders face in finding new heterotic patterns of maize. The main aim of a breeder is to obtain new hybrids that outperform the existing hybrids with respect to a number of traits. Grain yield is the most important trait in maize breeding programs and also it is the most complex quantitative trait determined by several yield components (Liu et al., 2008; Peng et al., 2011). Grain yield cannot be improved to a greater extent on its own, contribution of each component in the final expression of the complex character is very essential.

Heterosis is the superiority in performance of F1 hybrids over their parents (Stuber, 1994). Falconer and Mackay (1996) defined heterosis or hybrid vigour as the difference between the hybrid and the mean of the two parents. This difference is called mid-parent heterosis. Higher heterosis is associated with greater parental diversity. Therefore, assigning hybrids into heterotic groups is one of the fundamental pillars in breeding as it gives information on the extent of genetic diversity of parents (Amiruzzaman et al., 2010). The type of parents chosen determine the level of heterosis in maize (Ali et al., 2012), that is why it is important to select superior parents. Gurung et al. (2013) highlighted on the importance of studies on maize heterotic groups and patterns as they are very helpful in increasing breeding efficiency and exploitation of heterosis. Inbred lines that are distantly related can be crossed to exploit heterosis which can contribute to hybrid performance. Amiruzzaman et al. (2010) reported that standard heterosis for grain yield ranged from -17.60 to 9.71%. Similar results were also reported by Jawaharlal et al. (2012). This implies that selection can be done on the hybrids that performed better than the standard control.

Before introgression of genes coding for a trait of interest, it is essential to determine its heritability. Heritability can be expressed as narrow sense or broad sense (Smalley et al., 2004). In the narrow sense, heritability is measured by genetic variance due to additive genes divided by total phenotypic variance (Falconer, 1981). For the broad sense, heritability is

measured by total genetic variance divided by the total phenotypic variance (Falconer, 1981). Therefore, it is necessary to partition the observed variability into its heritable and non-heritable components. The information can be used to estimate genetic coefficient of variation, phenotypic coefficient of variation and heritability (Govindaraj et al., 2010). The knowledge of heritability can also be useful in identifying how much of an adaptive trait can be transferred to the progeny.

Knowledge on genetic diversity is very important in a maize breeding program. Data obtained on the genetic diversity of parents can be used to select appropriate germplasm for hybrid combinations (Prasanna, 2002). Cholastova et al. (2011) reported that it is very difficult to improve existing breeding strategies or to generate new breeding strategies if there is very limited information on genetic diversity. This is because selection of improved genotypes is dependent on the knowledge of already existing genetic variability in the breeding materials (Cholastova et al., 2011).

Information on correlation among traits is important in determining the degree and magnitude to which they are associated with yield potential. Correlation coefficients measure the mutual association between a pair of traits when more than two traits are involved, though they may not give a clear picture of the importance of each trait in determining grain yield (Sre kov et al., 2010). Some traits can influence grain yield through other traits. Therefore, there is need to perform path coefficient analysis since it gives clearer information on the direct and indirect effects on yield compared to correlation analysis (Sre kov et al., 2010). This enables breeders to decide between direct and indirect selection.

Therefore the objectives of this study were:

- (i) to determine heterosis in dent and flint maize hybrids
- (ii) to determine the association between genetic distances and phenotypic traits in dent and flint maize hybrids
- (iii) to determine the levels of variation and heritability for phenotypic traits in dent and flint maize hybrids.

2.1 Materials and methods

2.1.1 Germplasm

A total of 190 experimental dent and flint hybrids and two standard commercial maize hybrids were evaluated at Ukulinga (PAN67 and PAN6Q445B) and Cedara (PAN67 and PAN3Q740BR). The hybrids were developed by random crosses that were made among 60 inbred lines (dent and flint) at Ukulinga Research farm in 2012A (first season) (Khoza, 2012). The inbred lines are shown in the dendrogram Figure 2.1. The inbred lines are divided into two major groups A and B. Group A consists of inbred lines DXL19 to DXL101. Group B consists of inbred lines DXL24 to DXL162 (Figure 2.1). Group A consists of public lines namely MO17, PA2 and B73WX. Group B consists of public lines M162W and LP23 (Figure 2.1).

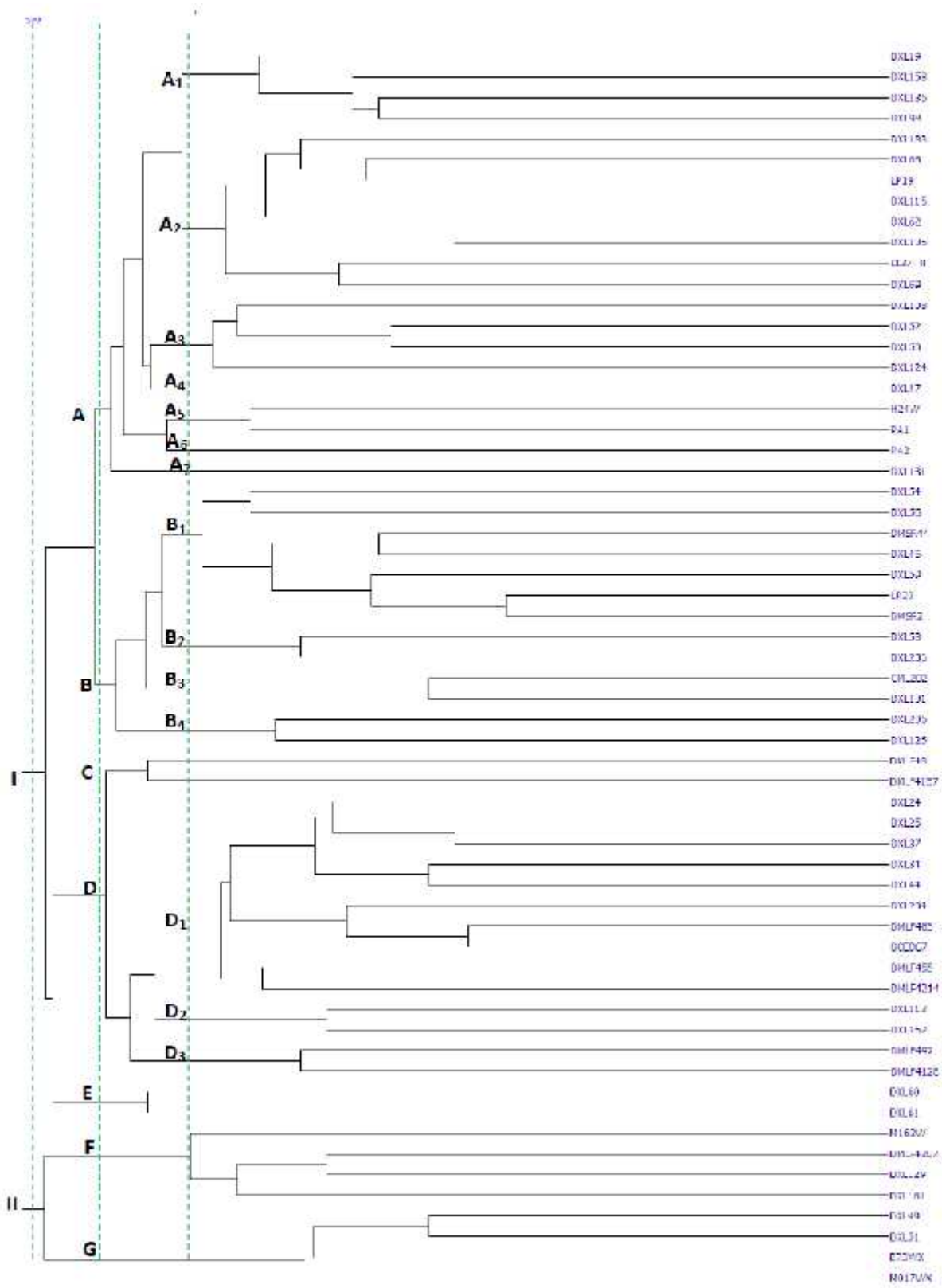


Figure 2.1: Dendrogram of 60 maize inbreds based on 29 molecular marker data (Khoza, 2012).

2.1.2 Experimental sites

The hybrids were evaluated across two sites in South Africa during the 2012/3 season. The sites used were Ukulinga Research Farm and Cedara Research Farm (Table 2.1). Ukulinga trial was planted on 5 November 2012 and Cedara trial was planted on 4 December 2012.

Table 2.1: Geographical coordinates and environmental conditions of study sites

Location	Latitude	Longitude	Altitude m.a.s.l	Total season	
				rainfall (mm)	temperature range (°C)
Ukulinga	29.67S	30.41E	809	676.17	11.99 -29.99
Cedara	29.54S	30.26E	1068	696.96	9.75 -27.89

2.1.3 Field trial design and management

Hybrids were evaluated at Ukulinga and Cedara Research Stations during the 2012/13 season. Experiments were laid out as augmented 10 x 19 alpha lattice designs at each site. The experimental hybrids were not replicated but the two control hybrids (checks) were replicated in all the 19 blocks at each site. The hybrids were planted at 90 cm and 75 cm between rows at Cedara and Ukulinga, respectively. The in-row spacing was 30 cm at both sites and plants were thinned to a single plant per station. The yields of the experimental hybrids were standardised to the usual 12.5% moisture content.

The experiments were conducted under rain fed conditions at both sites; the distribution of the monthly rainfall for the growing season is shown in Figure 2.2. The temperature data is shown in Figure 2.3. Fertilizer was applied as basal at planting in the form of compound (NPK) 2:3:4 at 250 kg ha⁻¹ (56 kg ha⁻¹ of N, 83 kg ha⁻¹ of P and 111 kg ha⁻¹ of K). Nitrogen fertilizer was applied at 4 weeks after crop emergence in the form of LAN (Lime Ammonium Nitrate, 28% N) at the rate of 250 kg ha⁻¹. The herbicides, Gramoxone, Dual, Basagran, and 2,4-D were applied to control weeds. This was augmented by hand weeding to keep the fields relatively clean of weeds throughout the season. Insecticide granules were applied in the

maize leaf whorls for stalk borer control. An insecticide, Karate, was applied to control cutworm at planting and seedling emergence.

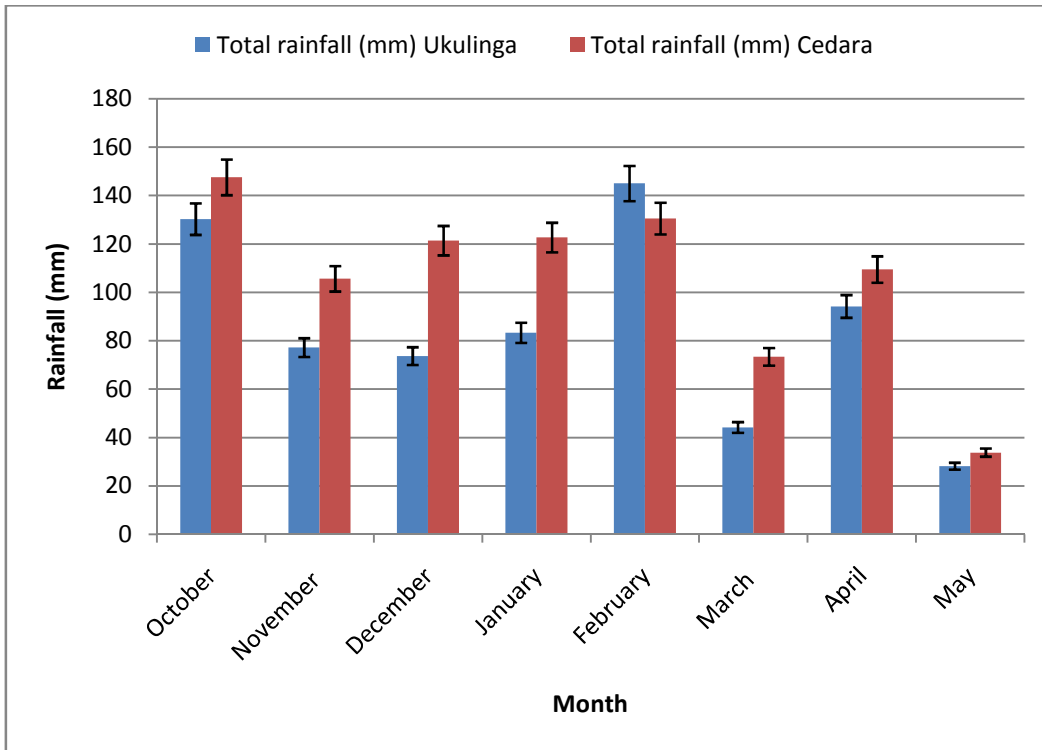


Figure 2.2: Total rainfall for Ukulinga and Cedara October 2012 to May 2013

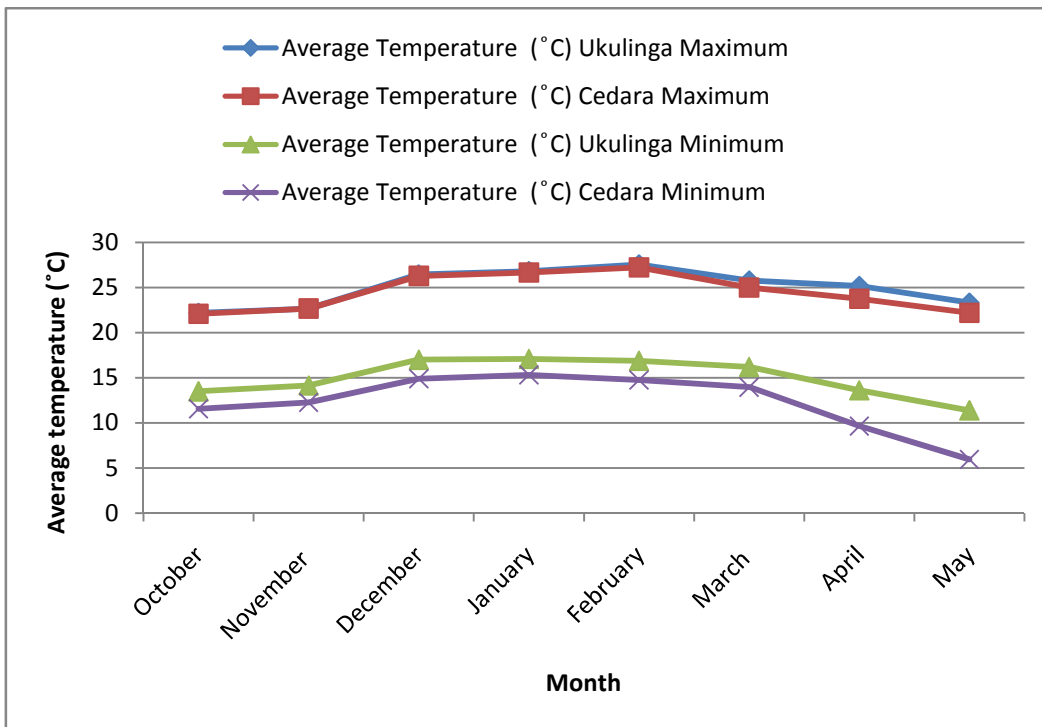


Figure 2.3: Average temperature of Ukulinga and Cedara October 2012 to May 2013

2.1.4 Data collection

The maize traits were measured following standard protocols of CIMMYT (Magorokosho et al., 2009):

- a) **Grain yield:** was measured as grain mass per plot adjusted to 12.5% grain at harvest
- b) **Plant height (cm):** measured as the distance between the base of plant to the insertion point of the top ear. It was measured when all the plants had flowered, since plants reach their maximum height at flowering.
- c) **Ear height (cm):** measured as height from ground level up to the base of the upper most ears bearing internodes.
- d) **Ear position:** measured as the ratio of ear height to plant height. Small values indicate low ear position and large values indicate high ear position.
- e) **Days to anthesis:** measured as the number of days when 50% of the plants shed pollen
- f) **Anthesis-silking interval (ASI):** determined by (i) measuring the number of days after planting when 50% of the plants shed pollen (anthesis date, AD) and show silks (silking date, SD).
- g) **Root lodging:** measured as percentage of the plants per plot which have their stem inclining by more than 45° .
- h) **Stem lodging:** measured as the percentage of plants per plot that have their stems broken below the ear.
- i) **Husk cover:** measured as percentage of plants with ears that are not completely covered by the husk.
- j) **Grain texture:** rated on a scale 1 (flint) to 5 (dent).
- k) **Grain moisture:** measured as percentage water content of grain measured at harvest

- l) **Ear prolificacy (EPP):** counted as number of ears with at least one fully developed grain divided by the number of harvested plants. An EPP of below 1.0 indicates partial bareness; an EPP of above 1.0 indicates ear prolificacy.
- m) **Plant aspect:** plant rating on a 1-5 scale, where 1 is excellent looking and 5 is very bad looking (high ear placement, too tall)
- n) **Ear aspect:** ear rating on a 1-5 scale, where 1 has desirable ear aspect and 5 has poorest ear aspect (rotten, too dent, small, uneven rows)
- o) **Ear diameter:** measured as the circumference of the middle part of an ear
- p) **Yield score:** yield score rating on a 1-5 scale, where 1 has desirable large ears and 5 poorest, small, not attractive
- q) **Stay green:** stay green rating in a 1-5 scale, where 1 has the desirable stay green colour and 5 lacks stay green trait
- r) **Number of plants:** number of plants harvested per plot

2.1.5 Data analysis

Prediction of mean performance of hybrids

The data was analysed using SAS version 9.3 (SAS Institute Inc, 2011) following the Proc Mixed and Proc GLM Models as described by Scott and Milliken (1993).

$$Y_{ijk} = \mu + B_i + C_j + X(C)_k + E_{ijk}$$

Where, Y_{ijk} = observed hybrid response

μ = overall trial mean

B_i = effect of the i^{th} block

C_j = effect of the j^{th} hybrid control (checks)

$X(C)_k$ = effect of the experimental hybrids within checks

E_{ijk} = random experimental error.

The block effects were treated as random while the hybrid main effects were considered fixed.

Estimation of genetic parameters

Genetic parameters were estimated for different traits on maize genotypes as follows:

Estimate of Standard Heterosis

Experimental hybrids were compared with performance of the two control (check) hybrids that were grown in the same block. The two control hybrids were grown in each block to facilitate standardisation of means of hybrids that were tested in different blocks. The estimates of heterosis for each experimental hybrid over the standard control were calculated (Hallauer and Miranda, 1981). In this case there were two control hybrids that were planted in each block and the mean of the two controls in each block was used in the calculation of standard heterosis as follows:

$$\text{Standard Heterosis (StdHET)} = \frac{F1 - MC}{MC} \times 100$$

The estimates of Better control heterosis were calculated using the following procedure (Hallauer and Miranda, 1981).

$$\text{Better control (BC) Heterosis} = \frac{F1 - BC}{BC} \times 100$$

Where,

F1 is the mean of experimental hybrids

MC is the mean of control hybrids

Standard heterosis was calculated on a block basis so that performance of hybrids that were grown in different blocks could be compared.

Estimate of Heritability

Variance component analysis was performed in SAS using the Proc Varcomp procedure. In this study any negative variance was treated as zero.

The estimates for heritability were calculated using the following procedure (Hallauer and Miranda, 1988)

$$H^2 = \frac{\sigma^2_g}{\sigma^2_g + \sigma^2_e} \times 100.$$

Where, σ^2_g = genotypic variance, σ^2_e = error variance. $\sigma^2_p = (\sigma^2_g + \sigma^2_e)$ = phenotypic variance.

Estimate of genetic gain (R)

The extent of genetic gain to be expected by selecting five per cent of the superior hybrids was calculated using the following formula (Singh and Chaudhary, 1979).

$$R = i H^2 \sigma_p$$

Where,

i = efficiency of selection which is 2.06 at 5% selection intensity

σ_p = phenotypic standard deviation

H = heritability in a broad sense

Genotypic and phenotypic coefficient of variation

The genotypic and phenotypic coefficient of variation was computed according to (Singh and Chaudhary, 1979) and expressed as percentage, as follows:

Genetic coefficient of variation: $GCV = \left(\frac{\sigma^2_g}{\bar{X}} \right) * 100$

Phenotypic coefficient of variation: $PCV = \left(\frac{\sigma^2_p}{\bar{X}} \right) * 100$

Where, σ^2_g = Genotypic variance, σ^2_p = Phenotypic variance and \bar{X} = general mean of the character.

Genetic distance

Genetic distance was estimated using data for inbreds which was sent to DNA landmarks for genotyping.

2.2 Results

2.2.1 Genetic variation

The two sites that were used in the study represented different environmental domains therefore results are reported on an individual site basis in addition to combined data. The analysis of variance for Ukulinga data is presented in Table 2.2. The mean squares due to checks were highly significant ($p < 0.01$) for most of the traits. The mean squares due to the test hybrids within checks were only significant ($p < 0.05$) for root lodging, yield score, ear aspect, plant aspect and hundred seed weight.

The mean squares due to the check hybrids at Cedara were highly significant ($p < 0.01$) for all the traits except root lodging (Table 2.3). The mean square values due to hybrids within checks were significant ($p < 0.05$) for most of the traits except root lodging, grain texture and grain moisture.

The analysis of variance for the combined sites data is presented in Table 2.4, and the mean squares for the check hybrids were highly significant ($p < 0.01$) for all the traits across the two sites. The same applies to hybrids within checks and the interaction between checks and sites. The interaction between sites and test hybrids within checks was highly significant ($p < 0.01$) for ear position, root lodging and stem lodging.

Table 2.2: Analysis of variance of 192 maize hybrids for quantitative traits at Ukulinga

Source	df	GYG	GYF	AD	ASI	PH	EH	EPO	RL	EL	HC	ED
..... Mean squares												
Block	18	0.25*	0.24*	0.04	2.42	0.58*	0.64	0.002	0.001	0.02	0.07	0.002
Check	2	5.17**	4.34**	0.02	4.35	0.71	1.11	0.001	0.014**	0.65**	0.04**	0.06**
X (C)	188	0.16	0.16	0.03	1.71	0.39	0.65	0.003	0.003*	0.06*	0.1	0.005
Error		0.11	0.1	0.02	2.51	0.24	0.38	0.002	0.001	0.02	0.06	0.004
R² (%)		96.41	96.61	96	89.83	96.73	96.67	93.89	97.05	97.61	95.87	95.91
CV (%)		12.81	11.54	1.59	-499.95	3.02	5.54	10.88	94.7	3.18	14.11	2.76

Source	df	EPP	SG	YS	TEX	GM	NP	EA	PA	HSDW	RY6Q
Block	18	0.08	0.17**	0.09*	0.03	0.002	0.003	0.07	0.07*	0.0002	0.02
Check	2	3.86**	0.37**	0.14*	0.64**	0.001	0.03**	0.65**	0.63**	0.002**	1.21**
X (C)	188	0.08	0.05	0.05*	0.08	0.002	0.009	0.09*	0.06*	0.0002*	0.04*
Error		0.05	0.05	0.02	0.05	0.03	0.003	0.04	0.03	0.00007	0.02
R² (%)		96.78	96.19	97.09	95.23	90.3	97.32	97.04	97.23	97.74	97.74
CV (%)		86.6	13.13	8.18	14.16	1.6	4.49	11.33	10.9	4.48	19.56

*, ** indicate level of significance at 5% and 1% respectively. C = control hybrids. X(C) = experimental hybrids (X) nested within control hybrids

GYG = grain yield. GYF = grain yield field. (GYF is the yield of cobs and GYG is the yield of shelled cobs). AD = anthesis date. ASI = anthesis silking interval. PH = plant height. EH = ear height. EPO = ear position. RL = root lodging. EL = ear length. HC = husk cover. ED = ear diameter. EPP = ear prolificacy. SG = stay green. YS = yield score. TEX = grain texture. GM = grain moisture. NP = number of plants. EA = ear aspect. PA = plant aspect. HSDW = hundred seed weight. RY6Q = control hybrid PAN6Q445B.

Table 2.3: Analysis of variance of 192 maize hybrids for quantitative traits at Cedara

Source	df	GYF	PH	EH	EPO	RL	SL	TEX	GM	EL	ED	SQRTgy
					Mean squares.....						
Block	18	1.28	218.23	170.86	0.002	6.15	87.58	0.53	0.28	1.15	0.07	0.04
Check	2	78.13**	4379.43**	12353.84**	0.17**	5.08	3180**	22.2**	65.67**	29.72**	0.4*	2.4**
X (C)	189	2.57*	385.75*	245.84*	0.004*	2.57	331.72**	0.63	1.23	5.39**	0.12*	0.08*
Error		1.02	142.5	107.79	0.0012	6.84	47.82	0.82	0.77	0.73	0.05	0.034
R² (%)		97.55	97.36	97.88	98.27	82.98	98.94	91.93	96.67	98.88	96.47	97.44
CV (%)		11.4	4.49	8.16	7.13	741.69	30.87	30.09	5.45	4.04	4.37	6.22

*, ** indicate level of significance at 5% and 1% respectively. C = control hybrids. X(C) = experimental hybrids (X) nested within control hybrids

GYF = grain yield field. PH = plant height. EH = ear height. EPO = ear position. RL = root lodging. SL = stem lodging. TEX = grain texture. GM = grain moisture. EL = ear length. ED = ear diameter. SQRTgy = square root for grain yield.

Table 2.4: Analysis of Variance of 192 maize hybrids for quantitative traits across two sites

Source	df	GYF	PH	EH	EPO	RL	SL	TEX	GM	EL	ED
Site	1	0.66	14.78	155.51	0.002	5420.83**	23407**	8.91*	662.55**	137.41**	18.15**
Block	18	6.23	314.15	209.46	0.002	35.13	43.78	0.49	0.17	1.34	0.07
Check hybrids	2	30.88*	3833.54**	5908.38**	0.05**	507.78**	1599.16**	27.06**	34.11**	64.16**	0.65**
X (C)	189	5.78*	605.60*	389.45 *	0.004**	108.40**	180.43**	1.01*	0.78*	8.24**	0.17**
Site*Check	2	204.34* *	1286.38*	6895.29**	0.07**	516.81**	1566.88**	2.47*	31.61**	13.09*	0.86**
Site*X (C)	188	2.58	328.46	216.03	0.002**	100.39**	173.57**	0.48	0.60*	2.21*	0.06
Error		3.86	296.17	192.42	0.001	25.95	30.54	0.61	0.34	1.46	0.06
R² (%)		91.78	93.18	94.45	95.84	97.74	98.81	91.78	98.9	97.01	95.77
CV (%)		23.63	6.5	11.19	8.07	83.95	49.22	26.84	4.04	5.94	4.89

*, ** indicate level of significance at 5% and 1% respectively; C = control hybrids. X(C) = experimental hybrids (X) nested within control hybrids. Site*X(C) = Site x experimental hybrids within control hybrids interaction.

GYF = grain yield field. PH = plant height. EH = ear height. EPO = ear position. RL = root lodging. SL = stem lodging. TEX = grain texture. GM = grain moisture. EL = ear length. ED = ear diameter.

2.2.2 Heritability

The heritability % was categorized as low, moderate and high in accordance with Robinson et al. (1949) as follows: 0-30%, Low; 30-60%, Moderate and >60%, High. The study revealed the whole range of low to high heritability estimates.

The broad sense heritability at Ukulinga ranged from 0 to 100% (Table 2.5). High broad sense heritability was exhibited by grain yield and ear diameter only. Whereas, grain textures, ear length, stay green, ear aspect and pant aspect recorded moderate heritability. Plant height, ear height, ear position, anthesis to silking interval, husk cover, number of plants, root lodging and grain moisture exhibited low heritability.

At Cedara broad sense heritability ranged from 0 to 100% (Table 2.6). High broad sense heritability was exhibited by grain yield, ear height, ear position and grain texture, while plant height, ear prolificacy and stem lodging data revealed moderate heritability. Root lodging, grain moisture, and ear length and ear diameter exhibited low heritability. There was no genetic variation for ear position, anthesis date, husk cover and grain moisture at Ukulinga hence heritability was zero, the same for Cedara where root lodging, grain moisture and ear length data displayed heritability of zero.

2.2.3 Genetic advance and coefficient of variation

There was a wide range for the genetic advance data at both sites. Genetic advance for Ukulinga data ranged from 0.00 to 9.31 with ear prolificacy exhibiting the highest genetic advance Table 2.5. Genetic advance for Cedara data ranged from 0.00 to 55.91 with ear height displaying the highest genetic advance (Table 2.6).

Phenotypic coefficient of variation was higher than genotypic coefficient variation for all of the traits at both sites. Genotypic coefficient of variation of test hybrids at Ukulinga ranged from -113.50 to 245.46 and for Cedara it ranged from 0.00 to 55.47. Phenotypic coefficient variation

of test hybrids for Ukulinga ranged from -425.63 to 245.46 and for Cedara it ranged from 7.51 to 500.00.

Table 2.5: Heritability estimates for quantitative traits of maize hybrids at Ukulinga

Trait	σ^2_g	Heritability (H^2) %	GA	GCV%	PCV%
Grain yield	9.57	100	6.37	44.81	44.81
Plant height	29.82	7.63	3.11	2.07	7.49
Ear height	15.31	5.3	1.86	3.24	14.07
Ear position	0.00	0.00	0.00	0.00	0.00
Anthesis date	0.00	0.00	0.00	0.00	3.84
Anthesis silking Interval	0.13	7.03	0.20	-113.5	-425.63
Root lodging	55.59	28.72	8.23	63.18	117.8
Ear length	2.38	50.22	2.26	7.89	11.17
Husk cover	0.00	0.00	0.00	0.00	36.45
Ear diameter	0.06	63.67	0.42	5.10	6.80
Ear prolificacy	20.47	100	9.31	245.46	245.46
Stay green	0.20	34.14	0.53	14.17	23.93
Yield score	0.08	12.78	0.21	7.52	21.76
Grain texture	0.42	47.13	0.91	23.24	33.60
Grain moisture	0.00	0.00	0.00	0.00	2.18
Number of plants	0.85	18.93	0.83	5.3	12.20
Ear aspect	0.44	38.6	0.85	20.27	32.87
Plant aspect	0.37	56.92	0.94	24.42	32.03

σ^2_g = genotypic variance. H^2 (%) = broad sense heritability. GA = genetic advance. GCV% = genotypic coefficient of variation. PCV% = phenotypic coefficient of variation.

Table 2.6: Heritability estimates for quantitative traits of maize hybrids at Cedara

Trait	σ^2_g	Heritability (H^2) (%)	GA	GCV%	PCV%
Grain yield	4.73	100.00	4.47	0.00	24.52
Plant height	260.34	64.60	26.72	4.50	7.57
Ear height	736.72	100.00	55.91	0.00	21.36
Ear position	0.01	100.00	0.21	0.00	20.83
Ear prolificacy	0.05	38.84	0.30	19.31	25.52
Grain texture	1.25	100.00	2.31	0.00	37.09
Stem lodging	179.94	53.80	20.27	55.47	81.62
Root lodging	0.00	0.00	0.00	0.00	500.00
Grain moisture	0.00	0.00	0.00	0.00	8.55
Ear length	0.00	0.00	0.00	0.00	11.15
Ear diameter	0.01	8.31	0.06	1.94	6.41

σ^2_g = genotypic variance. H^2 (%) = broad sense heritability. GA = genetic advance. GCV% = genotypic coefficient variation. PCV% = phenotypic coefficient variation.

2.2.4 Mean performance

There was a wide range on grain yield across the two sites. Top 10 hybrids that exhibited highest yield at Ukulinga are presented in Table 2.7, and the data ranged from 11.56 to 14.97 tha^{-1} . The yield for the bottom 10 at Ukulinga ranged from 1.24 to 3.11 tha^{-1} . Cedara yield of the top 10 hybrids is shown in Table 2.8 and the data ranged from 11.41 to 13.63 tha^{-1} . The yield for bottom 10 ranged from 2.72 to 5.11 tha^{-1} . Table 2.9 shows the means for the hybrids yield across the two sites, Ukulinga and Cedara, and the top 10 hybrids that performed well across both sites. The mean yield at both sites was 7.95 tha^{-1} .

Grain texture for most of the hybrids in top 10 was similar across the two sites. Most of the hybrids had grain texture of 3, meaning they were half dent and half flint. Ukulinga (Table 2.7) had seven hybrids that had grain texture of 3 and Cedara (Table 2.8) had six hybrids. Only one hybrid displayed a dent grain texture and two were flint at Ukulinga. At Cedara, there were two dent and two flint maize hybrids in the top 10.

GD ranged widely for the hybrids (0.19-0.83). Ukulinga had five hybrids with genetic distance above the trial mean, 0.51 (Table 2.7). In contrast to Cedara which only had one hybrid that was below the trial mean (Table 2.8). There was a slight difference on the genetic distance between the hybrids that were below the trial mean at both sites.

Considerable amount of heterosis was observed for most of the hybrids under study. Most of the hybrids that had high hybrid vigour across the two sites came from different heterotic patterns except for three hybrids 5, 142 and 143. Heterotic patterns were represented by group A and B. Both sites had five hybrids of the B*A heterotic patterns. Further, Ukulinga had two hybrids of A*B and two of B*B heterotic patterns, with only one hybrid of the A*A heterotic pattern (Table 2.7). Similarly, Cedara had three hybrids of the B*B heterotic pattern and only one hybrid of A*B and A*A (Table 2.8) in top 10.

All the hybrids in the top 10 at Cedara showed positive standard heterosis over the mean of control hybrids and the better control compared to Ukulinga, where one hybrid showed

negative heterosis over the mean of control hybrids and five hybrids showed negative heterosis against the better control hybrids. Standard heterosis over the mean control for Ukulinga for the whole ranged from -86.45 to 54.46% and over better control the range was from -89.90 to 52.27% (Table 2.7). Standard heterosis for Cedara ranged from -68.23 to 70.04% and heterosis over the better control ranged from -75.45 to 48.56% (Table 2.8).

In terms of parents that were constituents of the winning maize hybrids, DXL124 and DXL158 were predominant at both sites. DXL124 appeared five times in the top 10 at Cedara and once at Ukulinga and the opposite happened for DXL158 which appeared four times at Ukulinga and two times at Cedara. Further, DXL158 was predominant in the top 10 hybrids across the two sites. DXL124 only appeared once across the two sites. However there were parents that were predominant across the two sites though they were not predominant at individual sites, these include DXL126 and DMLF 485 (Table 2.9).

The importance of genotype x environment interaction effects was revealed because a different set of hybrids appeared in the top 10 at each site. There is only one hybrid 181 which appeared in the top 10 at both sites though at different positions. At Ukulinga it was on number 10 and on number three at Cedara. The rank correlation of the hybrids between the two sites was not similar meaning the difference was due to the genotype x environment interaction (Table 2.9).

Table 2.7: Grain yield mean, grain texture, heterosis and heterotic patterns of the top 10 maize hybrids at Ukulinga

Entry	Name	Pedigree	Parentage	Heterotic pattern*	GD	Grain Tex (score)	Grain Yield		Standard heterosis (%)	
							Mean (t/ha)	Rank	Over Mean of controls	Over Better control
150	13XH212	12UK10-30 X 12UK10-13	DXL126*DXL54	B*A	0.53	3	14.97	1	54.46	52.27
162	13XH231	12UK10-34 X 12UK10-35	DXL158*DXL161	A*B	0.49	2	13.63	2	44.71	18.22
160	13XH225	12UK10-32 X 12UK10-53	DXL131*DMLF485	A*B	0.58	3	12.72	3	34.58	2.70
26	13XH032	12UK10-39 X 12UK10-30	DXL206*DXL126	B*B	0.40	3	12.43	4	19.89	-1.62
35	13XH043	12UK10-46 X 12UK10-34	Tester 10* DXL158	A*A	-	4	12.24	5	29.99	3.92
179	13XH254	12UK10-5 X 12UK10-40	DXL37* DXL236	B*A	0.64	3	10.92	6	34.61	12.47
79	13XH109	12UK10-53*12UK10-29	DMLF485*DXL124	B*B	0.60	3	10.62	7	21.42	-24.48
123	13XH175	12UK10-2-*12UK10-34	DXL24*DXL131	B *A	0.49	2	10.57	8	-73.5	-78.14
166	13XH236	12UK10-35*12UK10-34	DXL161*DXL158	B*A	0.49	3	10.57	9	22.59	-1.99
181	13XH256	12UK10-6 X 12UK10-34	DXL44*DXL158	B*A	0.59	3	11.56	10	19.99	-4.07
Trial Mean					0.51		7.04			
Trial Min					0.19		1.24		-86.45	-89.90
Trial Max					0.83		14.97		54.45	52.27
Control-A							11.01			
Control-B							6.94			

*Heterotic grouping is indicated in Figure 2.1, - indicate missing data, grain texture score 1= flint and 5 = dent. GD = genetic distance.

Table 2.8: Grain yield mean, grain texture, heterosis and heterotic patterns of the top 10 maize hybrids at Cedara

Entry	Name	Pedigree	Parentage	Heterotic pattern*	GD	Grain Tex (score)	Yield		Standard heterosis (%)	
							Mean (t/ha)	Rank	Over Mean Control	Over Better control
143	13XH204	12UK10-29 X 12UK10-52	DXL124*DMLF45	B*B	0.52	3	13.63	1	63.91	29.26
142	13XH203	12UK10-29 X 12UK10-35	DXL124*DXL161	B*B	0.57	2	12.89	2	70.04	19.45
181	13XH256	12UK10-6 X 12UK10-34	DXL44*DXL158	B*A	0.59	3	12.65	3	49.84	28.16
41	13XH050	12UK10-29 X 12UK10-42	DXL124*CML202	B*A	0.54	3	12.43	4	47.00	14.65
161	13XH228	12UK10-33 X 12UK10-47	DXL136*TESTER9	A*A	-	4	12.28	5	49.84	21.53
5	13XH007	12UK10-52X 12UK10-29	DML455*DXL124	B*B	0.52	3	12.27	6	46.94	48.56
182	13XH258	12UK10-6 X 12UK10-9	DXL44*DXL49	B*A	0.53	3	12.21	7	42.52	7.90
128	13XH181	12UK10-2 X 12UK10-9	DXL24*DXL49	B*A	0.54	4	11.65	8	40.38	1.83
173	13XH244	12UK10-39 X 12UK10-34	DXL206*DXL158	B*A	0.49	2	11.46	9	35.87	15.15
159	13XH223	12UK10-32 X 12UK10-29	DXL131*DXL124	A*B	0.59	3	11.41	10	35.58	12.32
Trial Mean					0.51		8.85			
Trial Min					0.19		2.72		-63.23	-75.45
Trial Max					0.83		13.63		70.04	48.56
Control-A							10.46			
Control-B							6.49			

*Heterotic grouping is indicated in Figure 2.1, - indicates missing data, grain texture score 1= flint and 5 = dent. GD = genetic distance.

Table 2.9: Grain yield mean, grain texture, heterosis and heterotic patterns of the top 10 maize hybrids across two sites

Entry	Name	Pedigree	Parentage	Heterotic pattern*	GD	Grain Texture (score)	Mean Yield (t/ha)	Rank	Rank at Cedara	Rank at Ukulinga
150	13XH212	12UK10-30 X 12UK10-13	DXL126*DXL54	B*A	0.50	3	13.22	1	19	1
162	13XH231	12UK10-34 X 12UK10-35	DXL158*DXL161	A*B	0.50	2	12.63	2	34	2
58	13XH078	12UK10- 47 X 12UK10-34	Tester9*DXL158	A*A	-	3	12.23	3	38	14
26	13XH032	12UK10-39 X 12UK10-30	DXL206*DXL126	B*B	0.40	3	12.20	4	75	4
181	13XH256	12UK10-6 X 12UK10-34	DXL44*DXL158	B*A	0.60	3	11.74	5	3	11
79	13XH109	12UK10- 53 X 12UK10-29	DMLF485*DXL124	B*B	0.60	4	11.33	6	15	12
35	13XH043	12UK10-46 X 12UK10-34	Tester 10* DXL158	A*A	-	4	11.26	7	32	5
43	13XH054	12UK10- 40 X 12UK10-36	DXL236*DXL162	A*B	0.80	3	11.17	8	18	105
160	13XH225	12UK10-32 X 12UK10-53	DXL131*DMLF485	A*B	0.60	3	10.88	9	91	3
48	13XH063	12UK10- 44 X 12UK10-36	LP19*DXL162	A*B	0.70	2	10.79	10	37	95

Heterotic grouping is indicated in Figure 2.1, -indicates missing data, grain texture score 1= flint and 5 = dent. GD = genetic distance.

2.2.5 The frequency distribution of hybrids for yield and secondary traits

Four traits including grain yield that were significant at both sites were selected to observe their frequency distribution. Grain yield showed a continuous distribution in both environments. Grain yield was normally distributed at Cedara, however, at Ukulinga grain yield was skewed to the right meaning that most of the genotypes were low yielding (Figure 2.4). In addition, many genotypes at Cedara yielded between 6 t/ha and 12 t/ha. Furthermore, the distribution for ear length was continuous at both sites (Figure 2.5). Cedara had more hybrids with higher ear length compared to Ukulinga. Ear prolificacy at both sites were skewed to the right meaning there were many hybrids which had low prolificacy (Figure 2.6). Furthermore, grain moisture showed a continuous distribution at both sites (Figure 2.7).

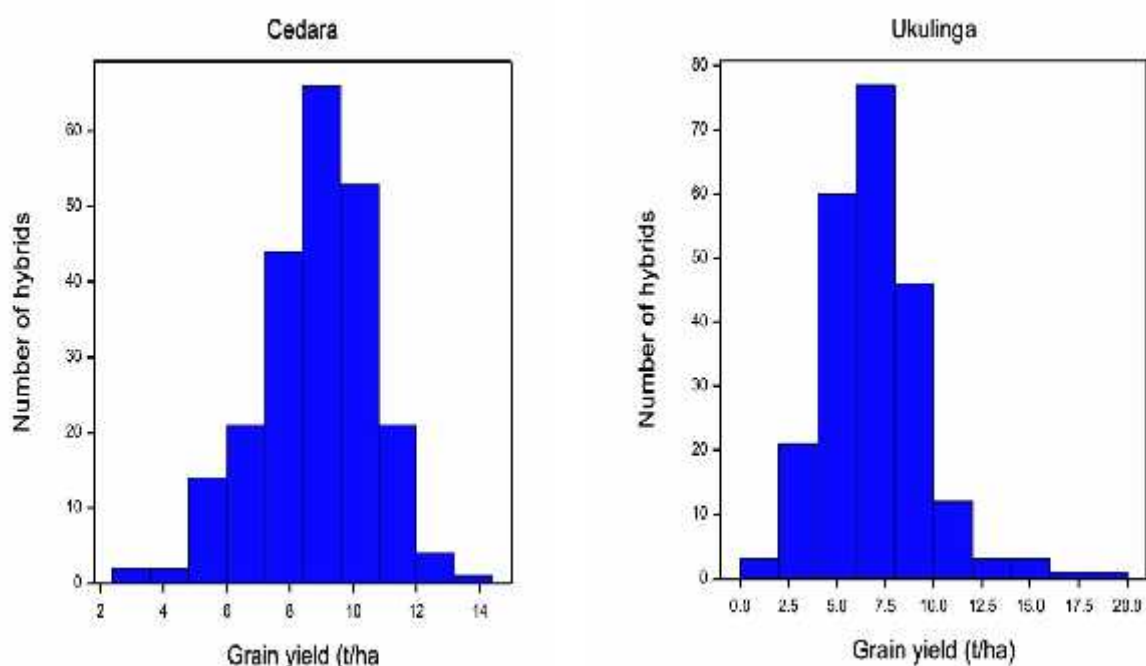


Figure 2.4: The histogram of grain yield for maize hybrids across two environments

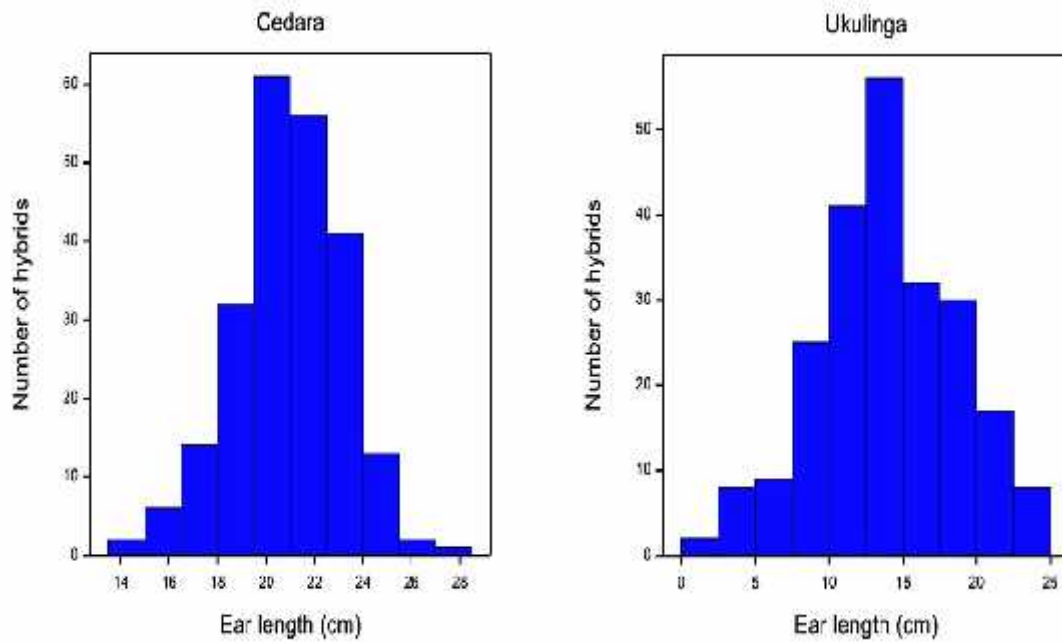


Figure 2.5: The histogram for ear length for maize hybrids across two environments

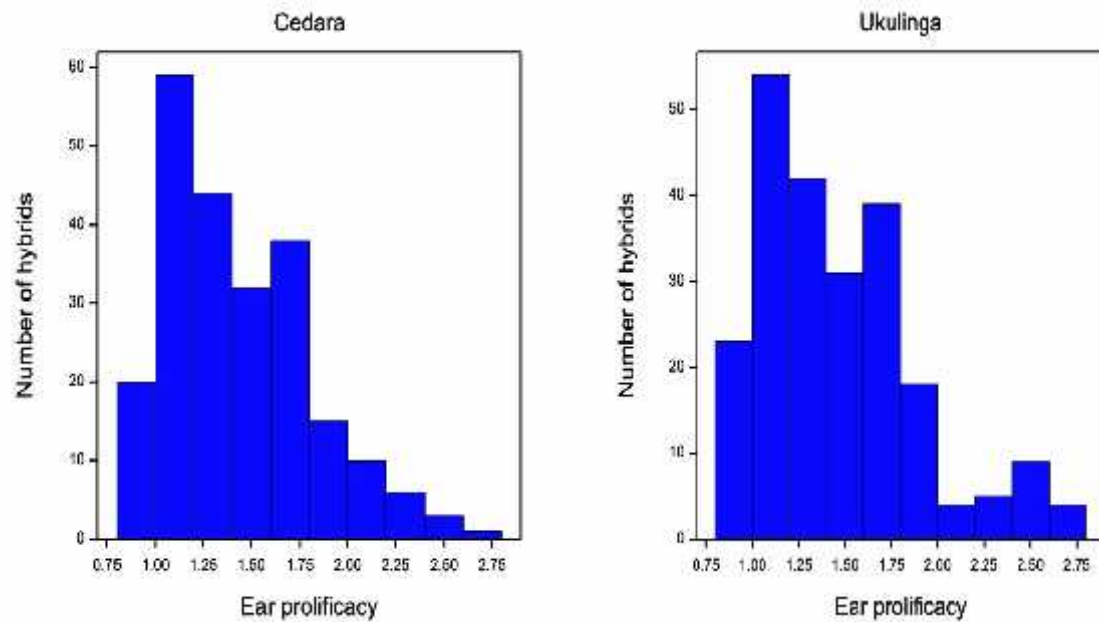


Figure 2.6: The histogram for ear prolificacy for maize hybrids across two environments

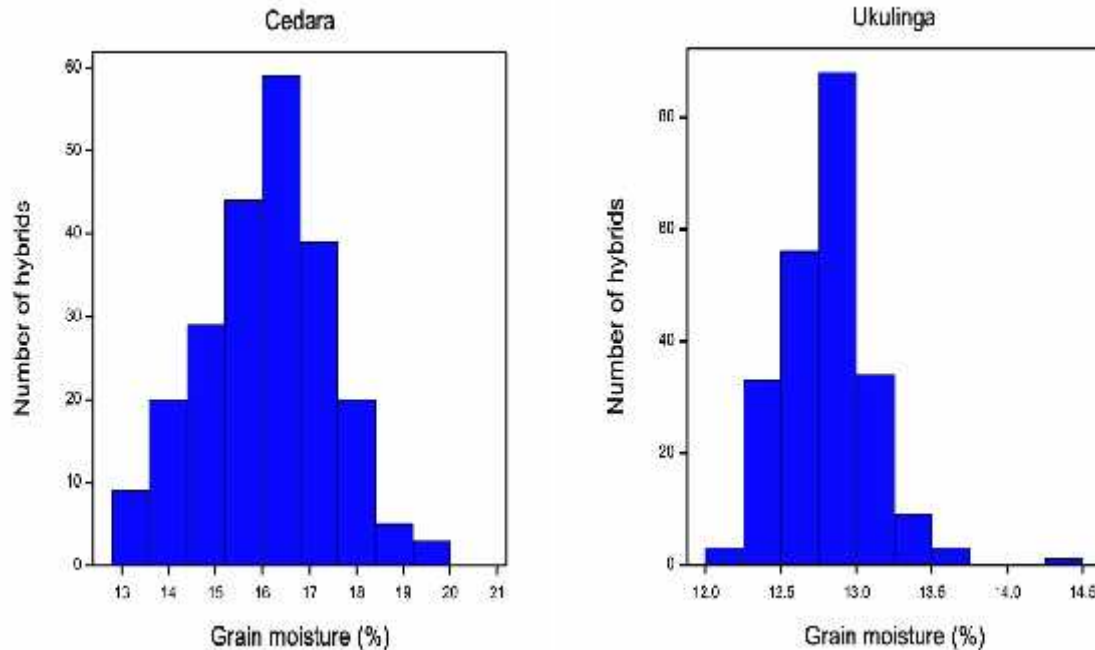


Figure 2.7: The histogram for grain moisture for maize hybrids across two environments

2.2.6 Relationships between phenotypic traits and genetic distance

The relationship between grain yield and genetic distance depended on the sites. Genetic distance was significantly ($p < 0.01$) and positively correlated with grain yield at Cedara. In sharp contrast, the grain yield data from Ukulinga does not show any significant correlation with genetic distance. Furthermore, there was not any significant ($p > 0.05$) relationship between secondary phenotypic traits and genetic distance at Ukulinga (Table 2.10). In contrast, there was significant correlation between genetic distances of the parents with secondary phenotypic traits at Cedara (Table 2.11). At Cedara, the data showed that correlation between genetic distance was positive and highly significant ($p < 0.01$) for ear prolificacy, grain texture and grain moisture.

2.2.7 Relationships among phenotypic traits

The study showed that there were significant correlations among phenotypic traits at both sites. Plant height, ear height, ear length, ear diameter, prolificacy, grain texture, number of plants and plant aspect showed highly significant ($p < 0.01$) and positive correlation with grain yield at Ukulinga (Table 2.10). Whereas, anthesis date, anthesis to silking interval, stay green, yield score and ear aspect exhibited highly significant ($p < 0.01$) and negative correlation with grain yield (Table 2.10). Similarly, at Cedara plant height, ear height, grain moisture, ear length and ear diameter were highly significant ($p < 0.01$) and positively correlated with grain yield (Table 2.11). Whereas only grain texture was significant ($p < 0.05$) and negatively correlated with yield (Table 2.11).

Table 2.10: Relationship of yield, secondary traits and genetic distance at Ukulinga

	GYF	GD	AD	ASI	PH	EH	EPO	RL	EL	HC	ED	EPP	SG	YS	TEX	MOI	NP	EA	PA
GYF	-																		
GD	0.01	-																	
AD	-0.32**	0.01	-																
ASI	-0.19*	-0.01	0.02	-															
PH	0.46**	0.04	-0.32**	-0.17*	-														
EH	0.39**	0.03	-0.17	-0.13	0.82**	-													
EPO	0.17	-0.02	0.03*	-0.05	0.37**	0.76**	-												
RL	-0.20*	-0.02	0.03*	0.07	0.10	0.21*	0.27**	-											
EL	0.53**	0.04	-0.15*	-0.12	0.41**	0.22*	-0.04	-0.24	-										
HC	0.00	0.00	-0.14*	-0.11	-0.02	0.01*	-0.03	-0.11	0.16*	-									
ED	0.22*	0.12	-0.22	0.03	0.25**	0.17*	0.02	-0.03	0.36**	0.13*	-								
EPP	0.56**	-0.10	-0.26**	-0.16	0.19*	0.20*	0.13	-0.15*	0.06	-0.02*	-0.16*	-							
SG	-0.46**	0.17	0.12	0.06	-0.21*	0.15*	-0.05	0.25*	-0.38**	-0.15	-0.31**	-0.35**	-						
YS	-0.65**	-0.07	0.45**	0.14	-0.48**	-0.31**	-0.08	0.08	-0.53**	0.06*	-0.32**	-0.29**	0.29**	-					
TEX	0.15**	-0.03	-0.20*	-0.06	0.12	-0.01	-0.12	-0.16*	0.18*	0.03	0.07	0.15*	-0.04	-0.13	-				
GM	0.09	0.03	0.22*	0.02	-0.06	-0.02	0.04	0.05	0.01	-0.16*	0.11	-0.05	-0.14*	-0.01	-0.25*	-			
NP	0.33**	-0.04	0.03	0.01	-0.04	0.05	0.11	0.03	-0.08	-0.12	-0.17*	0.04	0.23*	0.09	0.02	0.01	-		
EA	-0.62**	-0.13	0.14	0.10	-0.40**	-0.26**	-0.04	0.15	-0.58**	0.17*	-0.31**	-0.11	0.17*	0.58**	-0.08	-0.18*	-0.09	-	
PA	0.23*	-0.10	-0.04	-0.17	0.46**	0.53**	0.35**	0.20*	0.17*	0.11	0.20*	0.16*	-0.24*	-0.08	0.03	0.04	-0.06	-0.13	-

*, ** indicate level of significance at 5% and 1% respectively

GYF = grain yield field. GD = genetic distance. AD = anthesis date ASI = anthesis silking interval. PH = plant height. EH = ear height. EPO = ear position. RL = root lodging. EL = ear length. HC = husk cover. ED = ear diameter. EPP = ear prolificacy. SG = stay green. YS = yield score. TEX = grain texture. GM = grain moisture. NP = number of plants. EA = ear aspect. PA = plant aspect.

Table 2.11: Relationship of yield, secondary traits and genetic distance at Cedara

	Grain yield	Genetic distance	Plant height	Ear height	Ear position	Root lodging	Stem lodging	Ear prolificacy	Grain texture	Grain moisture	Ear length	Ear diameter
Grain yield	-											
Genetic distance	0.31**	-										
Plant height	0.39**	-0.01	-									
Ear height	0.32*	0.01	0.42**	-								
Ear position	-0.21	0.01	-0.20*	0.46**	-							
Root lodging	-0.13	-0.10*	0.12	0.16	0.04	-						
Stem lodging	-0.01	0.03	-0.01	0.18*	0.20*	0.09	-					
Ear prolificacy	-0.18	-0.21*	0.01	0.04	0.06	-0.04	-0.04	-				
Grain texture	-0.29*	0.01	-0.02	-0.15	-0.15	-0.2	-0.12	0.13	-			
Grain moisture	0.43**	0.23*	0.09	0.05	-0.03	0.13	0.24*	-0.30*	-0.34**	-		
Ear length	0.33**	0.14	0.29*	-0.02	-0.18*	-0.1	-0.08	-0.39**	0.05	0.26*	-	
Ear diameter	0.40**	0.30**	0.05	0.07	0.03	0.06	0.16	-0.45**	-0.09	0.35**	0.41**	-

*, ** indicate level of significance at 5% and 1% respectively

2.2.8 Path analysis: direct and indirect contribution of secondary traits to grain yield

Direct effects were more influential compared to the indirect effects at both sites. Regression data showing levels of significance for direct effects at Ukulinga is presented in Table 2.12. Regression of yield on anthesis date was significant at ($p < 0.05$). Regression of yield ear length, stay green and yield score were significant at 1% and lastly ear prolificacy and ear aspect was highly significant ($p < 0.0001$) (Table 2.12).

Ukulinga data for path analysis are presented in Table 2.14. At Ukulinga, ear prolificacy had the highest direct and positive effects on grain yield followed by ear height, ear length, and grain texture and grain moisture. Whereas, ear aspect had the highest direct negative effect on grain yield followed by yield score, stay green and anthesis date. Ear position had the highest indirect positive effect on grain yield through ear height; followed by plant height through ear height; and plant aspect via ear height. Stay green trait displayed the highest indirect negative effects on grain yield through ear prolificacy; followed by yield score via ear prolificacy; and anthesis date through ear prolificacy.

All the direct effects at Cedara were significant ($p < 0.01$; $p < 0.0001$) except for grain texture (Table 2.13). Path analysis for Cedara data is presented in Table 2.15. Ear position had the highest direct positive effects on grain yield followed by plant height, ear diameter, ear length, and grain moisture and ear prolificacy. Ear height had the highest direct negative effect on yield followed by grain texture. Ear height had the highest indirect positive effects on grain yield through ear position followed by grain texture through ear height and grain moisture via ear position. Plant height had the highest indirect negative effects on grain yield through ear height followed by grain texture via ear position and grain moisture through ear height.

Table 2.12: Regression data showing levels of significance for direct effects off secondary traits at Ukulinga (dent and flint hybrids)

Trait	Parameter estimate	Standard error	t-value	P-value
Anthesis date	-0.0898	0.0413	-2.17	0.0308*
Plant height	-0.0987	0.2716	-0.36	0.7166
Ear height	0.2231	0.4788	0.47	0.6418
Ear position	-0.0741	0.3045	-0.24	0.8079
Ear length	0.1599	0.0475	3.36	0.0009**
Ear diameter	-0.0395	0.0415	-0.95	0.3433
Ear prolificacy	0.4316	0.0418	10.32	<0.0001***
Stay green	-0.1399	0.0451	-3.1	0.0022**
Yield score	-0.1452	0.0509	-2.86	0.0047**
Grain texture	0.0589	0.0362	1.62	0.1058
Grain moisture	0.0471	0.0370	1.27	0.2044
Ear aspect	-0.3490	0.0468	-7.46	<0.0001***
Plant aspect	-0.0419	0.0414	-1.01	0.3128
Hundred seed weight	-0.0167	0.0392	-0.43	0.6708

*, ** and *** indicate significance at 5%, 1% and 0.01% respectively

Table 2.13: Regression data showing levels of significance for direct effects off secondary traits at Cedara (dent and flint hybrids)

Trait	Parameter estimate	Standard error	t-value	P-value
Plant height	0.8528	0.2355	3.62	0.0004**
Ear height	-0.2742	0.4370	-2.50	0.0130*
Ear position	0.2635	0.4031	2.79	0.0058**
Ear prolificacy	0.1692	0.0578	2.93	0.0038**
Grain texture	-0.0936	0.0599	-1.56	0.1198
Grain moisture	0.2563	0.0594	4.32	<0.0001***
Ear length	0.2434	0.0596	4.08	<0.0001***
Ear diameter	0.2148	0.0585	3.67	0.0003**

*, ** and *** indicate significance at 5%, 1% and 0.01% respectively

Table 2.14: Direct (underlined and bold) and indirect effects of different secondary traits on maize yield at Ukulinga. (n=227; R² = 0.77)

Grain yield component	AD	PH	EH	EPO	CL	CW	Ear prolificacy (EPP)*	SG	YS	TEX	GM	EA	PA	HSDW	Total correlation to grain yield
Anthesis date (AD)	<u>-0.09*</u>	0.03	-0.04	0.00	-0.02	0.01	-0.11	-0.02	-0.07	-0.01	0.01	-0.05	0.00	0.00	-0.35
Plant height (PH)	0.03	<u>-0.10</u>	0.18	-0.03	0.06	-0.01	0.08	0.03	0.07	0.01	0.00	0.14	-0.02	0.00	0.43
Ear height (EH)	0.01	-0.08	<u>0.22</u>	-0.06	0.03	-0.01	0.09	0.02	0.04	0.00	0.00	0.09	-0.02	0.00	0.34
Ear position (EPO)	0.00	-0.04	0.19	<u>-0.07</u>	0.00	0.00	0.07	0.01	0.01	-0.01	0.00	0.02	-0.02	0.00	0.16
Ear length (EL)	0.01	-0.04	0.05	0.00	<u>0.16**</u>	-0.01	0.03	0.05	0.08	0.01	0.00	0.20	-0.01	0.00	0.53
Ear diameter (ED)	0.02	-0.03	0.04	0.00	0.06	<u>-0.04</u>	-0.07	0.05	0.05	0.00	0.01	0.11	-0.01	0.00	0.18
Ear prolificacy (EPP)*	0.02	-0.02	0.04	-0.01	0.01	0.01	<u>0.43***</u>	0.05	0.04	0.01	0.00	0.04	-0.01	0.00	0.61
Stay green (SG)	-0.01	0.02	-0.03	0.00	-0.06	0.01	-0.15	<u>0.14**</u>	-0.04	0.00	-0.01	-0.06	0.01	0.01	-0.45
Yield score (YS)	-0.04	0.05	-0.07	0.01	-0.08	0.01	-0.12	-0.04	<u>-0.15**</u>	-0.01	0.00	-0.20	0.00	0.00	-0.64
Grain texture (TEX)	0.02	-0.01	0.00	0.01	0.03	0.00	0.06	0.01	0.02	<u>0.06</u>	-0.01	0.03	0.00	0.00	0.20
Grain moisture (GM)	-0.02	0.01	0.00	0.00	0.00	0.00	-0.02	0.02	0.00	-0.01	<u>0.05</u>	0.06	0.00	0.00	0.07
Ear aspect (EA)	-0.01	0.04	-0.06	0.01	-0.09	0.01	-0.05	-0.02	-0.08	0.00	-0.01	<u>-0.35***</u>	0.01	0.00	-0.62
Plant aspect (PA)	0.00	-0.04	0.12	-0.03	0.03	-0.01	0.07	0.03	0.01	0.00	0.00	0.04	<u>-0.04</u>	0.00	0.18
Hundred seed weight (HSDW)	0.02	-0.02	0.03	-0.01	0.03	-0.01	0.10	0.07	0.03	0.01	0.00	0.03	-0.01	<u>-0.02</u>	0.27

*EPP = ear prolificacy, i.e., ear prolificacy. *, ** and *** indicate level of significance at 5%, 1% and 0.01% respectively.

Table 2.15: Direct (underlined and bold) and indirect effects of different secondary traits on maize yield at Cedara. (n=228; R²= 0.45)

Grain yield component	Plant height	Ear height	Ear position	Ear prolificacy (EPP)*	Grain texture	Grain moisture	Ear length	Ear diameter	Total correlation to grain yield
Plant height	<u>0.82**</u>	-0.43	-0.13	0.01	0.00	0.03	0.06	0.03	0.39
Ear height	0.33	<u>-0.26*</u>	0.89	0.03	0.05	0.06	0.01	0.01	0.32
Ear position	-0.10	-0.46	<u>0.25**</u>	0.03	0.06	0.04	-0.03	-0.01	-0.22
Ear prolificacy	0.03	-0.16	0.14	<u>0.19**</u>	0.01	-0.02	-0.08	-0.11	0.00
Grain texture	-0.02	0.36	-0.39	-0.01	<u>-0.15</u>	-0.10	0.02	-0.01	-0.29
Grain moisture	0.13	-0.28	0.22	-0.02	0.07	<u>0.21***</u>	0.03	0.06	0.43
Ear length	0.24	-0.04	-0.13	-0.07	-0.01	0.03	<u>0.23***</u>	0.11	0.35
Ear diameter	0.08	-0.04	-0.02	-0.08	0.01	0.05	0.09	<u>0.28**</u>	0.37

*EPP = ear prolificacy, i.e., ear prolificacy. *, **and ***indicate level of significance at 5%, 1% and 0.01% respectively.

2.3 Discussion

2.3.1 Genetic variation

The study indicated the presence of substantial genetic variability among the test hybrids but this depended on the environment. The results are in accordance with Mitrovic et al.(2012) who reported that the highest percentage of variation in their study was explained by environment. The variability among hybrids for grain yield contributing characters in maize was also reported by Solanke et al. (2013). This is an indication that genetic variation for the traits under study was present, which is good for breeding because breeding depends on availability of variation. The observed large genetic variation is in line with a number of studies that have reported genetic variation for economic traits such as plant and ear height (Bello et al., 2012; Kage et al., 2013), root and stem lodging (Bello et al., 2012; Prasanna, 2012), grain moisture at physiological maturity (Reid et al., 2010; Filipenco et al., 2013), and grain yield (Bello et al., 2012). Therefore, most traits have the potential to be included in the selection index for hybrids.

2.3.2 Heritability

The data from both sites exhibited high heritability for grain yield data. These results are in contrast with Asghar and Mehdi (2010) who reported heritability of 38% for grain yield. This is because of the different genotypes that were used and also different environments and different management of the experiments. However, high levels of heritability for both yield and secondary traits have also been reported by other researchers which are consistent with the current study. Nadagoud (2008) reported heritability estimates ranging from 89% for the number of kernels per ear to 97% for grain yield. Aminu and Izge (2012) reported the highest heritability estimates of 99% for grain yield. This implies that genetic variation was higher than environmental variation for grain yield at both sites, which means genetic variation contributed more than the environmental variation to performance of test hybrids. High heritability estimates in maize for grain yield were also reported by Lorenzana and Bernardo (2008). Therefore, there would be a high response to selection for grain yield.

Estimate of heritability for secondary traits varied significantly between the sites indicating the role of genotype x environment effects for conditioning these traits. Ear height was highly

heritable at Cedara compared to Ukulinga; this implies that at Cedara there was large genetic variation. These results are in contrast with Aminu and Izge (2012) who reported moderate heritability of 58% for ear height. Although high genetic variation was observed at Cedara, the results imply that ear height is highly influenced by the environment as revealed by very low heritability at Ukulinga. This suggests that this trait might not respond to selection in some environments. These results are in line with the findings of Lorenzana and Bernardo (2008). Plant height was highly heritable at Cedara compared to Ukulinga indicating the influence of environmental effects on this trait. This is in line with results from Aminu and Izge (2012) who reported heritability of 60% on plant height, and Ullah et al. (2013) who reported heritability of 69% for plant height. Shahrokhi and Ebrahimi (2013) and Ullah et al. (2013) have reported low, medium and high estimates of heritability in different plant traits in different environments and working with different hybrids. Higher and relatively moderate broad sense heritability of the traits revealed that variations were transmissible and potential for developing high yielding varieties through selection of desirable plants in succeeding generations exists.

2.3.3 Genetic advance and variation

Although grain yield had the highest heritability, its genetic advance at both sites was low. This implies that there is still need to improve the performance of yield. Ear height and plant height had the highest genetic advance at Ukulinga, and this shows that there was high genetic improvement for these traits. There is need to improve the performance of other traits. The higher phenotypic coefficient of variation over genotypic coefficient of variation of most traits across the two environments indicates the significant genotype x environment interactions in the expressions of these traits. Similar results were reported by Manigopa and Rameswar (2012) who found that PCV was higher than GCV for many traits. Bello et al. (2012) reported that there were significant differences among the genotypes for measured characters; PCV was slightly higher than GCV for all of the characters suggesting the presence of environmental influence to some extent in the expression of these characters. Nadagoud (2008) reported that PCV was comparatively higher than GCV suggesting the influence of the environmental factor on all characters.

2.3.4 Standard heterosis

A considerable amount of heterosis was observed for most of the hybrids under study with Ukulinga having higher yields compared to Cedara for the top 10 hybrids. Cedara is a medium altitude and a higher yielding environment compared to Ukulinga which is a low altitude and low yielding environment (Table 2.1). However, in this study Ukulinga had higher yields than Cedara due to the difference in the planting dates at the sites. Ukulinga was planted earlier than Cedara. The plants did not fix energy over the whole season at Cedara which compromised grain yield potential. At Ukulinga, maximum grain yield was obtained because of earlier planting which is consistent with Tsimba et al. (2013).

The level of standard heterosis that was observed in these test hybrids justified the development of commercial crosses in maize, because some of the top 10 hybrids were superior to the commercial control hybrids. High heterosis on grain yield shown in this study is consistent with a number of previous studies which showed standard heterosis of 55% for grain yield (Sundararajan and Kumar, 2011). Mandal et al. (2009) reported a range of -8.24% to 189.69% for grain yield. Such potential of maize crosses for commercial exploitation are in line with studies conducted by Avinashe et al. (2013), Premlatha and Kalamani (2010), Singh and Gupta (2009) and Sundararajan and Kumar (2011). Crosses that were outstanding in respect of grain yield can be exploited commercially, after critical evaluation for their superiority for performance and stability across different locations. Therefore, the top 10 hybrids will be advanced in the breeding programme.

2.3.5 Genetic distance versus yield

Results from the study showed that the hybrids obtained from crossing parents with larger genetic distance (average GD > 0.50) gave the highest yields at both sites. It was observed that, in general, all the top 10 hybrids involved parents with greater than 50% genetic distance. These results are consistent with Boonlertnirun et al. (2012) who reported high heterosis for parents that had large genetic distances (GD>71%). This has also been echoed by Flint-Garcia et al. (2009) that closely related genotypes show much lower heterosis than more distantly related genotypes. However, in the current study, there were a few exceptions especially at Ukulinga where three of the top 10 hybrids involved parents with less than 50% GD. Additionally, the study indicated that the relationship between yield and genetic distance

would depend on the environment. In the current study, the correlation between yield and GD was almost independent (0.005) at Ukulinga whilst it was highly correlated at Cedara. It seems therefore that the relationship between genetic distance and yield of hybrids was complicated by genotype x environment interaction. Some researchers such as George et al. (2011), have found that there is no relationship between GD and grain yield.

2.3.6 Grain texture versus yield

Most hybrids that gave high yield in this study had a grain texture of 3 meaning they were half dent and half flint. These results are in contrast with Boppenmaier et al. (1992), who reported that high yields were obtained for dent x dent crosses than dent x flint patterns. Grain texture was significant and positively correlated with grain yield ($p < 0.05$) at Ukulinga and at Cedara as well ($p < 0.01$). Path analysis on grain texture at both sites showed that there was no significant correlation between grain yield and grain texture. This implies that grain texture cannot be used as direct selection for grain yield.

2.3.7 The frequency distribution of hybrids for yield and secondary traits

The results revealed that the frequency distribution patterns of grain yield and secondary traits varied from one environment to another due to significant genotype x environment effects. Grain yield for Ukulinga was skewed to the left, meaning most hybrids were low yielding though there were a few exceptions which had very high yields hence its top 10 yielded more than Cedara. Furthermore, many genotypes at Cedara were prolific whereas many genotypes at Ukulinga were not prolific; this indicated that ear prolificacy on these genotypes was unstable; its expression depended on the conditions of the environment. These results showed that the majority of the hybrids at Ukulinga were not prolific, indicating that the trait cannot be selected to improve grain yield. Grain moisture was continuous at both sites indicating that there was no GEI. All the histograms showed continuous distribution of these quantitative traits, which confirms involvement of many genes in their control in hybrids.

2.3.8 Relationships between phenotypic traits and genetic distance

Plant height, ear height, ear length, ear diameter, prolificacy, grain texture, number of plants and plant aspect showed highly significant and positive correlation with grain yield at Ukulinga. This implies that increasing expression of these traits can positively influence grain yield. These results are concurrent with Selvaraj and Nagarajan (2011) who reported that plant height, ear height and ear length and grain weight showed significant positive association with yield. The results are in line with Akbar et al. (2008) and Bocanski et al. (2009) but in contrast with Sreckov et al. (2011). The difference between the results is mainly due to difference in germplasm used and the test environments. Hence, there is need to evaluate genotypes under different environments to determine the effects of genotype x environment on the correlation between yields and secondary traits. Anthesis date, anthesis to silking interval, ear aspect (Ukulinga), showed significant and negative correlation with yield. These results show that a negative correlation on the anthesis date is favoured to obtain early maturing hybrids and also that targeting these traits would be effective for indirect selection of grain yield. These results are in contrast with Selvaraj and Nagarajan (2011) who reported that anthesis date and silking date showed positive non-significant association with grain yield.

In terms of parentage, the inbred line DXL124 dominated the top 10 hybrids at both sites meaning that it has potential for a good combining ability with other inbred lines, so in future studies DXL124 can be used as a potential tester to exploit specific combining ability. This also applies to DXL158 which proved to be combining well with other inbred lines due to its dominance in the top 10 hybrids. Since both DXL124 and DXL158 might have good combining ability with many other lines it might be prudent to test a cross of the two lines for specific combining ability, because they belong to different heterotic groups, DXL158 is from A and DXL124 is from B clusters.

2.3.9 Path coefficient analysis

Path coefficient analysis was used to partition the relationships between secondary traits and yield into direct and indirect effects. Ear prolificacy, ear height, ear length, and stay green traits had high significant direct effects on grain yield at Ukulinga, except for ear height. This is in line with the study carried out by Selvaraj and Nagarajan (2011) which revealed that

direct selection for ear length is effective for yield improvement since it revealed positive and significant relationship with grain yield. The same author stated that, the positive direct and indirect effects of a trait on grain yield make it possible for its exploitation in selection under specific conditions implying the role of GEI.

The Cedara site revealed the highest direct positive effect of plant height (0.82) on grain yield of hybrids, which is consistent with Pavan et al. (2011a) who reported high and positive direct effects of plant height and ear height on grain yield. The results are consistent with Khalili et al. (2013) who reported the direct effect of plant height (-0.82) on grain yield though with a negative magnitude. The present study suggests that traits such as ear height, prolificacy and ear diameter can be used as primary selection criterion when breeding for grain yield for target environments that are represented by Ukulinga since their positive direct effects on grain yield are least affected by indirect traits. Ear position, plant height, ear length and ear diameter should be used as target traits to improve maize grain yield for environments that are represented by Cedara.

Indirect effects were ranked according to Lenka and Mishra, (1973) as follows, 0.00 to 0.09 negligible, 0.10 to 0.19 low, 0.20 to 0.29 moderate and 0.30 to 0.99 high path coefficients. At Ukulinga negligible indirect effects on grain yield were exhibited by anthesis date, ear position, ear diameter, plant aspect and hundred seed weight. Plant height and yield score had low indirect effects and only ear aspect had high indirect path coefficient values. At Cedara only grain texture and ear height had low and moderate indirect effects on grain yield.

2.4 Conclusion

From the foregoing, it can be concluded that,

- The study indicated the presence of substantial genetic variability among the test hybrids but this depended on the environment
- Grain yield was highly heritable at all sites, indicating that direct selection for yield would be effective to obtain suitable hybrids with high yield potential in environments that were

represented by these sites. The results also showed that most secondary traits were genetically controlled but there was no genetic variation for some of them.

- The association between yield and genetic distance depended on the environment indicating that the relationship was affected by genotype x environment interaction effects; because grain yield of hybrids was positively correlated with genetic distance at one site but there was no correlation at the other site.
- In this study there were some hybrids that were in the top 10 but they had negative heterosis relative to the mean of control hybrids and the better control hybrid, meaning that these hybrids were not superior to the control hybrids. Thus for advancement in the breeding program, one would recommend selection of hybrids 150, 162 and 179 at Ukulinga. In a stark contrast, all the hybrids in the top 10 at Cedara can be selected because they performed better than the control hybrids.
- Hybrids that were in the top 10 for grain yield potential at Ukulinga were not the same as the hybrid set at Cedara, implying that there was a significant G x E interaction. However, there was an exception of the hybrid 181 which was in the top 10 for yield potential at the two sites though at different positions. Selection of this hybrid can be recommended because it displayed some level of stability and combined high stability with high productivity, which is a desired combination.
- The lines DXL124 and DXL158 dominated parentage of the top 10 hybrid rank for yield qualifying them as potential testers for specific combining ability in future studies.
- In this study flint x flint hybrids performed well as they appeared in the top 10, which is not consistent with previous findings. All the patterns of dent x dent, dent x flint and flint x flint were represented in the top 10 indicating that grain texture could not be readily used to discriminate hybrids for yield potential in this set of hybrids.
- The new heterotic patterns that performed better than the control hybrids can be utilized in heterosis breeding to enhance yield potential of maize hybrids. However, these hybrids must be tested in many different environments to check on their stability because the study also confirmed complications of selection due to significant genotype x environment interaction effects.
- The main significant direct factors contributing to yield were ear prolificacy, ear length and ear diameter, indicating that direct selection for these traits would be effective to improve grain yield of hybrids. Therefore, these traits can be used as primary selection criteria for grain yield in the respective environments.
- Indirect effects of secondary traits on yield were generally low and negligible; indicating that indirect selection of these traits would not improve yield potential of hybrids.

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Heterosis and Path Coefficient Analysis in Popcorn Experimental Hybrids

Abstract

Popcorn (*Zea mays* Everta) is a popular snack food in Sub-Saharan Africa but there are no locally bred hybrids in South Africa. There is limited genetic information about popcorn breeding which compromises efficiency of variety development. The objectives of the study were to determine heterosis between popcorn hybrids, to estimate correlation, direct and indirect effects of secondary traits on grain yield in popcorn hybrids. A total of 119 experimental hybrids and one standard control were evaluated at two sites. The experiments were laid out as 10x12 alpha lattice designs with two replications at each site. The data was analysed using mixed models in SAS. A considerable amount of mid-parent heterosis was observed for most traits under study, thus experimental hybrids which performed better than the parents would be advanced. For example, a heterosis value of 410% was realised for yield. From the study, it was observed that most of the traits were associated with grain yield both directly and indirectly. This indicated that selection for any one of these yield attributing traits will lead to increase in the other traits, and finally boosting grain yield. The main direct factors contributing to yield were ear prolificacy, number of plants, ear height, ear length and shelling percentage, indicating that direct selection for these traits would be effective to improve grain yield of hybrids. These traits can be considered as important quantitative traits in popcorn improvement programmes and seemingly direct selection for these traits would be rewarding.

Key words: correlation, mid-parent heterosis, path coefficient analysis, popcorn.

3 Introduction

Maize is one of the most important cereals grown in the world for food security, whereas popcorn is a common snack worldwide. Popcorn (*Zea mays* L. Var. *Everta*) industry has been increasing continuously throughout the world and is becoming accepted in South Africa. Popcorn is an extreme form of flint maize; it has a very hard endosperm and a small portion of soft starch. It is characterized by its ability to pop when heat is applied (Saavedra et al., 2013). Regardless of its importance, genetic improvement of popcorn is hindered by limited genetic varieties (Vijayabharathi et al., 2009). Increasing yield potential of popcorn in South Africa is still a challenge to plant breeders because there are a few varieties that are adapted to local conditions (Silva et al., 2011). There is not even a single popcorn hybrid that has been developed in South Africa since 1954 forcing farmers to rely on imported seed which is not adapted to local conditions (Josephson, 1954). Therefore, the current study aimed at identifying new popcorn hybrids that are adapted to production conditions in South Africa.

Generally popcorn has a narrow genetic base. The narrow genetic base creates genetic vulnerability which leads to increased susceptibility of crops to biotic and abiotic stress factors (Singh, 1993). This is undesirable for popcorn breeding programs because in order to create productive hybrids, a certain level of parental divergence is needed (Dandolin et al., 2008). Therefore, there is need to broaden the popcorn genetic base in future breeding efforts because heterosis depends on levels of genetic divergence between the parents.

Ali et al. (2012) defined heterosis as the superiority of an F1 that is produced by crossing two genetically different individuals over the mean of its parents. Therefore, there is need to develop new hybrids that exceed the performance of existing hybrids in yield and quality (Premlatha and Kalamani, 2010). Reif et al. (2005) reported that information on heterosis is very important as it enables breeders to group germplasm into different heterotic groups to exploit heterosis and improve on the performance of hybrids. George et al. (2011) reported that knowledge of the potential of a cross to produce highly heterotic hybrids can guide the choice of appropriate parental lines so that only promising hybrids are constructed. In maize, appreciable percentages of heterosis for yield were observed by several authors such as Dandolin et al. (2008); Avinashe. (2013); Jajwar and Chakraborty (2013).

Unfortunately, the components that contribute to high yield potential in popcorn germplasm are not known. Grain yield is a complex character and can be determined by several components which reflect positive and negative effects. Therefore, it is important to examine the contribution of each secondary trait to determine its influence on yield (Khazaei et al., 2010). This can be achieved by using path coefficient analysis. Path coefficient analysis provides an effective means of partitioning correlation coefficients into direct and indirect pathways. This leads to a critical examination of specific factors that produce a given correlation which can be successfully employed in formulating an effective selection programme in maize breeding (Salahuddin et al., 2010). Dallo et al. (2010) reported that path coefficient analysis is commonly used in crop breeding studies to establish the relationship between grain yield and its contributing components. The knowledge will be used to devise an effective breeding program for popcorn hybrids.

Therefore the objectives of the study were:

- i. to determine heterosis between popcorn hybrids
- ii. to determine the effect of secondary traits on grain yield in popcorn hybrids

3.1 Materials and methods

3.1.1 Germplasm

A total of 120 experimental hybrids were planted at Cedara Research Station and Ukulinga Research Farm during the 2011/2012 summer season. Hybrids were developed from 87 inbred lines. The crosses between inbred lines were made at random in 2010, depending on synchrony of anthesis and silking. Consequently only 119 hybrids managed to produce adequate seed for evaluation in trials. The commercial hybrid P618 was used as the control.

3.1.2 Field trial design and management

Experiments were laid in 10x12 alpha lattice designs with two replications at each site. Plants were spaced 30 cm apart within the rows and 90 cm between the rows. The plots were 5 m

long. The trials were planted on 22 November 2011 at Ukulinga Research Farm and 1 December 2011 at Cedara Research Station.

The fertilizer 2:3:4 was applied at the rate of 250 kg ha^{-1} at planting (56 kg ha^{-1} of N, 83 kg ha^{-1} of P and 111 kg ha^{-1} of K). Topdressing was done at 6 weeks by applying LAN (28%) at the rate of 250 kg ha^{-1} . Standard cultural practices for maize were applied including hand planting, use of herbicides and insecticides. Trials at both sites were rain fed.

3.1.3 Data collection

The following traits were measured as described in Chapter 2, grain yield, plant height, ear height, ear position, root lodging, stem lodging, grain moisture, days to anthesis, days to silking, ear prolificacy, ear length and number of plants per plot. In addition the following traits were measured following standard protocols used at CIMMYT (Magorokosho et al., 2009).

a) **Days to silking:** measured as the number of days after planting when 50% of the plants in the plot have silks 2-3 cm long.

b) **Ear length:** measured in centimetres from the base to the tip of the ear

3.1.4 Data analysis

Data was analysed using Proc GLM procedure in the SAS version 9.3 (SAS Institute Inc, 2011). The model used for data analysis across sites is as follows:

$$Y_{ij} = \mu + B_i + T_j + E_{ij}$$

Where, Y_{ij} = observed yield response

μ = grand mean

B_i = effect of the i^{th} block, $i = 1 \dots 2$

T_j =effect of the j^{th} treatment, $j = 1 \dots 120$

E_{ij} = random experimental error

Path analysis and correlation analysis were performed in SAS version 9.3 (Scott and Milliken, 1993).

Heterosis was calculated as:

$$\text{Mid Parent Heterosis (MPH\%)} = \frac{F_1 - MP}{MP} \times 100$$

Where,

F_1 = F1 hybrid performance

MP = the average performance of parents $\frac{P_1 + P_2}{2}$

In which P1 and P2 are the performance of the two parents (Fehr, 1991).

3.2 Results

3.2.1 The frequency distribution for yield and secondary traits

Four traits that were significant at both sites were selected and their distribution frequencies are shown in Figure 3.1 to Figure 3.4. Grain yield showed continuous distribution at both sites; however grain yield was also skewed to the right meaning most hybrids were low yielding (Figure 3.1). Grain yield, ear length, and ear prolificacy were continuous at both sites. Number of plants at Cedara was skewed to the left implying that there were many hybrids.

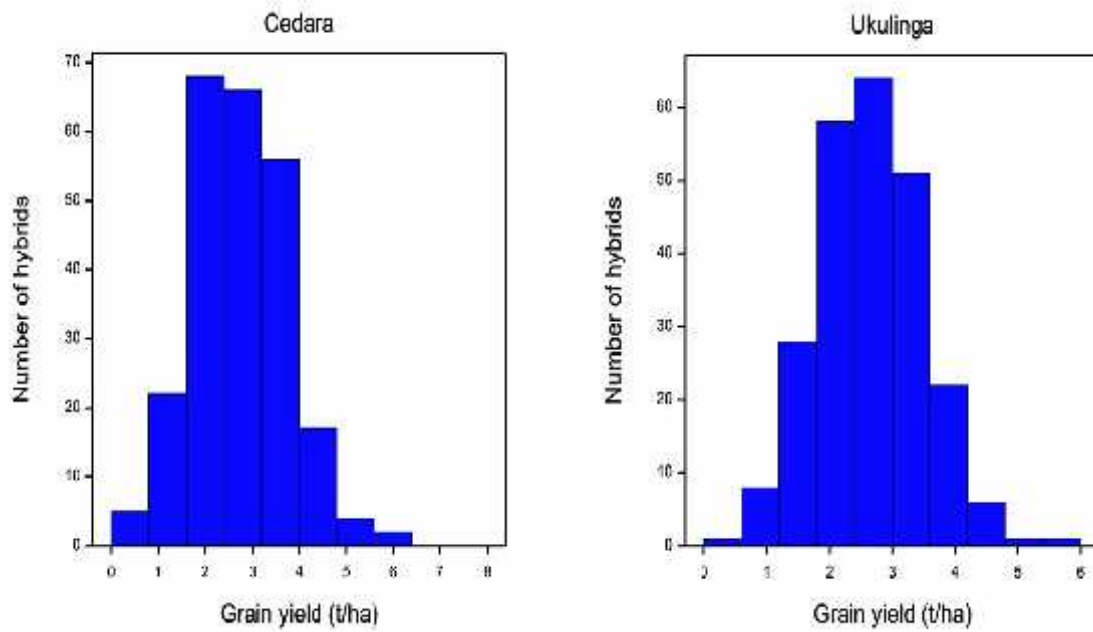


Figure 3.1: The histogram of grain yield for popcorn hybrids across two environments

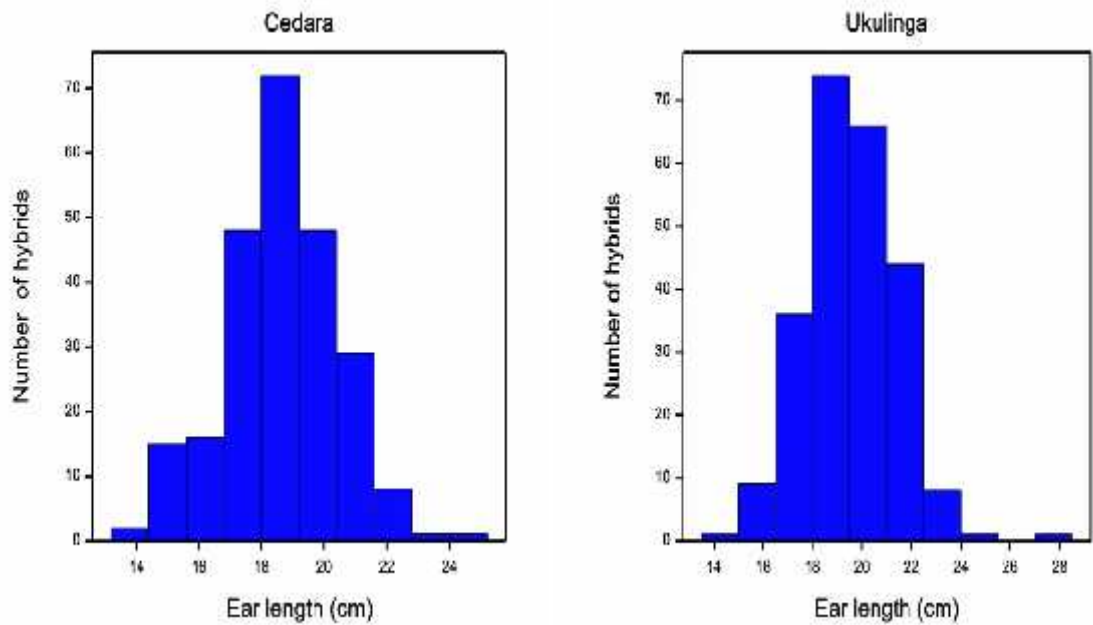


Figure 3.2: The histogram of ear length for popcorn hybrids across two environments

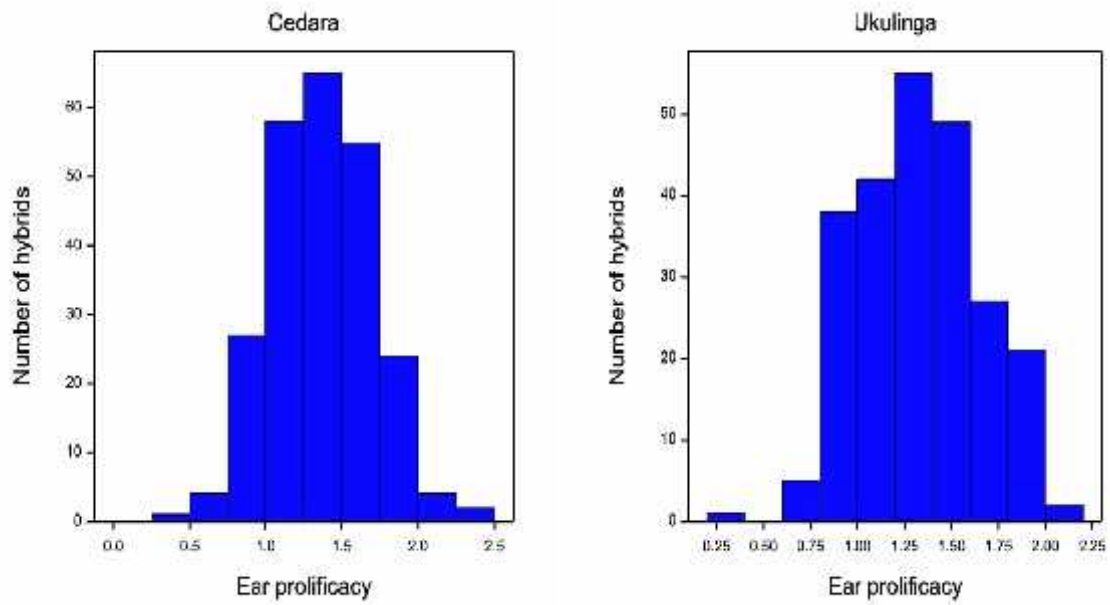


Figure 3.3: The histogram of ear prolificacy for popcorn hybrids across two environments

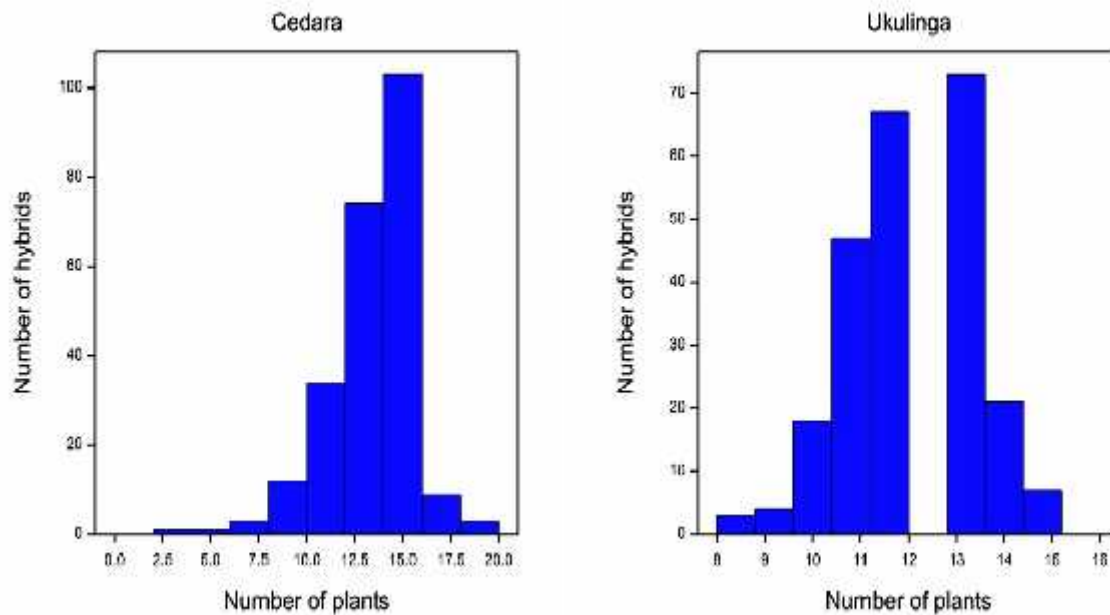


Figure 3.4: The histogram of number of plants for popcorn hybrids across two environments

3.2.2 Genetic variation and Mid-parent heterosis

There was wide genetic variation among hybrids for yield and secondary traits. Descriptive statistics for Cedara are shown in Table 3.1. There was a considerably high level of heterosis in the study. MPH% was estimated for Cedara only because at Ukulinga inbreds were not planted. MPH% was estimated for 12 traits (Table 3.2) and only top 10 hybrids which had high MPH% for grain yield are presented. The bottom five which had lowest MPH% are shown (Table 3.3). There was a wide variation for heterosis for all traits measured.

Table 3.1: Descriptive statistics for hybrid traits

Trait	Mean	Standard Deviation	Summation	Minimum	Maximum
Grain yield (t ha ⁻¹)	2.74	0.97	657.64	0.54	6.07
Ear length (cm)	18.67	1.78	4480.00	14.00	24.50
Plant height (cm)	230.03	24.88	55208.00	157.00	290.00
Ear height (cm)	113.33	20.64	27199.00	59.00	191.00
Number of plants	13.83	2.13	3320.00	4.00	20.00
Grain moisture (%)	14.56	0.95	3495.00	12.80	18.90
Days to anthesis	71.13	1.94	17071.00	67.00	77.00
Days to silking	70.20	1.97	16848.00	66.00	76.00
Ear position	0.49	0.06	117.94	0.31	0.68
Shelling percentage	0.73	0.12	175.39	0.00	1.69
Ear prolificacy	1.39	0.32	332.90	0.38	2.33
Root lodging (%)	9.79	14.97	2349.00	0.00	91.67
Stem lodging (%)	40.41	25.77	9699.00	0.00	100.00

Table 3.2: Heterosis for grain yield and secondary traits of the top 10 hybrids

Germplasm		GYG	EL	PH	EH	NP	GM	DA	DS	ASI	SH%	RL	SL	
Hybrid	Female parent	Male parent	MPH%	MPH%	MPH%	MPH%	MPH%	MPH%	MPH%	MPH%	MPH%	MPH%	MPH%	
30	32	19	410.61	36.08	7.84	24.61	10.34	16.94	-6.71	-6.80	0.00	10.10	353.00	-5.46
37	35	32	398.81	40.07	16.71	29.63	3.70	20.82	-5.41	-5.48	0.00	9.61	-79.26	28.72
38	3	4	398.72	21.92	7.40	24.59	7.14	19.91	-5.41	-5.48	0.00	16.84	-62.66	55.56
42	37	9	339.87	21.15	12.88	18.99	16.67	12.03	-4.73	-4.79	0.00	68.54	-83.59	0.88
22	18	49	326.93	30.36	29.80	32.97	-12.90	18.82	-4.03	-5.37	0.00	1.48	72.23	66.88
21	18	8	326.36	38.10	27.03	27.27	-13.79	28.46	-7.38	-7.48	0.00	7.47	-100.00	-63.51
76	53	19	316.19	25.24	21.05	25.14	3.57	15.92	-0.69	-0.70	0.00	11.43	40.61	-72.09
78	54	28	309.46	16.03	12.35	8.33	-6.90	10.16	-2.78	-5.48	-200.00	16.88	-100.00	-2.80
58	46	19	304.67	27.87	25.84	14.29	0.00	15.00	-5.41	-5.48	0.00	6.21	-100.00	-18.18
77	54	18	292.68	25.00	12.41	31.47	-6.67	12.75	-6.04	-7.38	0.00	18.63	-70.61	177.52

GYG = grain yield. EL = ear length. PH = plant height. EH = ear height. NP = number of plants. GM = grain moisture. DA = days to anthesis. DS = days to silking. ASI = anthesis silking interval. SH% = shelling percentage. RL = root lodging. SL = stem lodging. MPH% = Mid Parent Heterosis.

Table 3.3: Heterosis data for grain yield and secondary traits of the bottom five hybrids

Germplasm			GYG	EL	PH	EH	NP	GM	DA	DS	ASI	SH%	RL	SL
Hybrid	Female parent	Male parent	MPH%	MPH%	MPH%	MPH%	MPH%	MPH%	MPH%	MPH%	MPH%	MPH%	MPH%	MPH%
52	43	80	11.80	2.60	26.28	38.17	-6.45	7.54	0.72	0.73	0.00	5.30	107.15	2.15
103	72	47	10.54	16.21	0.00	14.44	-21.88	17.89	-5.92	-6.00	0.00	2.02	105.12	163.73
117	71	47	8.37	38.18	20.25	49.09	-62.5	22.13	-3.97	-4.03	0.00	0.83	-33.33	-25.00
35	34	57	-9.47	13.92	0.00	-19.66	-9.68	2.78	0.00	0.00	0.00	-6.11	-100.0	-10.1
100	70	74	-38.55	-6.08	-2.05	4.09	-28.57	14.17	-3.85	-2.60	-100.0	3.51	-100.0	0.00

GYG = grain yield. EL = ear length. PH = plant height. EH = ear height. NP = number of plants. GM = grain moisture. DA = days to anthesis. DS = days to silking. ASI = anthesis silking interval. SH% = shelling percentage. RL = root lodging. SL = stem lodging. MPH% = Mid Parent Heterosis.

3.2.3 Relationship among phenotypic traits

There was significant correlation among phenotypic traits at both sites. At Ukulinga, grain yield was significant ($r = 0.34$, $p < 0.01$) and positively correlated with ear prolificacy, plant height, ear height, shelling percentage, number of plants and ear length (Table 3.4). There was a negative correlation between grain yield and days to silking, root lodging and stem lodging though the relationship was not significant ($p > 0.05$). Days to silking was more significantly ($p < 0.01$) and positively correlated with days to anthesis also ear position was significantly ($p < 0.01$) and positively correlated with ear height.

At Cedara grain yield was significantly ($p < 0.01$) and positively correlated with ear height, grain moisture, plant height and ear prolificacy. Stem lodging was significant ($p < 0.01$) and negatively correlated with grain yield (Table 3.5). Days to anthesis and days to silking had weak and negative correlations with grain yield. Days to silking was significant ($p < 0.01$) and positively correlated with days to anthesis also ear position was significant ($p < 0.01$) and positively correlated with ear height.

Table 3.4: Relationship among phenotypic traits at Ukulinga Research Farm (n=120)

	Grain yield	Ear length	Plant height	Ear height	Number of plants	Grain moisture	Days to anthesis	Days to silking	Ear position	Shelling %	Ear prolificacy	Root lodging	Stem lodging
Grain yield	-												
Ear length	0.43**	-											
Plant height	0.47**	0.29*	-										
Ear height	0.45**	0.08	0.74**	-									
Number of plants	0.42**	0.03	0.05	0.09	-								
Grain moisture	0.20*	-0.01	0.06	0.14	-0.01	-							
Days to anthesis	0.03	-0.12	0.14	0.43**	0.08	0.13	-						
Days to silking	-0.03	-0.14	0.10	0.39**	0.04	0.14	0.98**	-					
Ear position	0.31*	-0.09	0.35*	0.88**	0.09	0.16	0.50**	0.48**	-				
Shelling percentage	0.40**	-0.04	-0.01	0.07	0.20	0.17	0.04	0.20	0.10	-			
Ear prolificacy	0.52**	0.02	0.43**	0.56**	-0.04	0.23*	0.28*	0.27*	0.49**	0.08	-		
Root lodging	-0.07	-0.01	-0.01	0.02	-0.01	0.16	0.20*	0.24*	0.03	0.03	0.06	-	
Stem lodging	-0.15	-0.22*	0.09	0.21	-0.12	-0.11	0.26*	0.27*	0.23*	-0.12	0.20*	0.05	-

*, ** indicate level of significance at 5% and 1% respectively

Table 3.5: Relationship among phenotypic traits at Cedara Research Farm (n=120)

	Grain yield	Ear length	Plant height	Ear height	Number of plants	Grain moisture	Days to anthesis	Days to silking	Ear position	Shelling percentage	Ear prolificacy	Root lodging	Stem lodging
Grain yield	-												
Ear length	0.34*	-											
Plant height	0.51**	0.36**	-										
Ear height	0.56**	0.25*	0.79**	-									
Number of plants	0.34*	0.01	0.10	-0.01	-								
Grain moisture	0.55**	0.31*	0.50**	0.45**	0.11	-							
Days to anthesis	-0.04	0.05	-0.08	0.08	-0.10	-0.01	-						
Days to silking	-0.05	0.02	-0.10	0.01	-0.11	-0.02	0.98**	-					
Ear position	0.38**	0.06	0.33*	0.84**	-0.11	0.25*	0.08	0.09	-				
Shelling percentage	0.04	-0.19*	-0.21*	-0.15	0.10	-0.22	0.01	0.01	-0.03	-			
Ear prolificacy	0.51**	-0.02	0.35**	0.43**	-0.22*	0.15	0.03	0.03	0.37**	-0.05	-		
Root lodging	0.10	0.13	0.13	0.23	0.01	0.26	0.05	0.06	0.24*	-0.02	-0.01	-	
Stem lodging	-0.37**	-0.27*	-0.32*	-0.19*	-0.01	-0.46**	0.01	-0.01	-0.02	0.18**	-0.13	-0.20	-

*, ** indicate level of significance at 5% and 1% respectively

3.2.4 Path coefficient analysis of secondary traits

Regression data showing levels of significance for direct effects at Ukulinga are shown in Table 3.6. Direct effects were more influential on yield compared to the indirect effects at both sites. Ear length, number of plants, shelling percentage and ear prolificacy were the only direct effects that were significant ($p < 0.0001$) with grain yield at Ukulinga (Table 3.6). Ear position, via ear height, exhibited the highest positive indirect effects on yield followed by plant height via ear height, ear prolificacy via ear height and days to silking via ear position followed by days to anthesis via days to silking, days to anthesis via ear position and days to silking via ear position.

Regression data showing levels of significance for direct effects at Cedara are shown in Table 3.7. Ear length, number of plants, grain moisture and ear prolificacy were significant at ($p < 0.0001$). Shelling percentage was significant at ($p < 0.01$) and stem lodging was significant at ($p < 0.05$). Path analysis results are presented in Table 3.9. Highest indirect positive effects were exhibited by ear position via ear height followed by plant height via ear height, ear height via ear prolificacy and ear position via ear prolificacy. Stem lodging, via grain moisture, showed highest negative indirect effects on yield followed by days to silking through days to anthesis, number of plants through ear prolificacy and ear height via plant height.

Table 3.6: Regression data showing levels of significance for direct effects at Ukulinga

Trait	Parameter	Standard	t-value	P-value
Ear length	0.3471	0.0502	6.91	<0.0001***
Plant height	-0.2027	0.3353	-0.60	0.5467
Ear height	0.6685	0.6596	1.01	0.3131
Number of plants	0.3458	0.0480	7.21	<0.0001***
Grain moisture	0.0666	0.0489	1.36	0.1760
Days to anthesis	0.2328	0.2228	1.04	0.2984
Days to silking	-0.3801	0.2234	-1.70	0.0918
Ear position	-0.3889	0.4699	-0.83	0.4097
Shelling %	0.2795	0.0478	5.84	<0.0001***
Ear prolificacy	0.4538	0.0572	7.94	<0.0001***
Root lodging	-0.0624	0.0484	-1.29	0.1999
Stem lodging	-0.0811	0.0509	-1.59	0.1139

*, ** and *** indicate level of significance at 5%, 1% and 0.01%

Table 3.7: Regression data showing levels of significance for direct effects at Cedara

Trait	Parameter Estimate	Standard error	t-value	P-value
Ear length	0.2328	0.0536	4.34	<0.0001***
Plant height	-0.1220	0.3478	-0.35	0.7265
Ear height	0.2722	0.5923	0.46	0.6468
Number of plants	0.4096	0.0505	8.11	<0.0001***
Grain moisture	0.2989	0.0616	4.85	<0.0001***
Days to anthesis	-0.1265	0.2593	-0.49	0.6268
Days to silking	0.0922	0.2601	0.35	0.7238
Ear position	-0.0045	0.3860	-0.01	0.9907
Shelling percentage	0.1644	0.0497	3.31	0.0013**
Ear prolificacy	0.4835	0.0557	8.68	<0.0001***
Root lodging	-0.0744	0.0506	-1.47	0.1444
Stem lodging	-0.1325	0.0558	-2.37	0.0194*

*, ** and *** indicate level of significance at 5%, 1% and 0.01%

Table 3.8: Direct (underlined and bold) and indirect effects of different traits in popcorn at Ukulinga (n = 120; R² = 0.78)

Trait	Ear length	Plant height	Ear height	Number of plants	Grain moisture	Days to anthesis	Days to silking	Ear position	Shelling percentage	Ear prolificacy	Root lodging	Stem lodging	Total correlation to grain yield
Ear length	<u>0.35***</u>	-0.11	0.08	0.01	0.00	-0.03	0.06	0.06	-0.01	0.01	0.00	0.02	0.43
Plant height	0.10	<u>-0.39</u>	0.78	0.02	0.00	0.04	-0.04	-0.23	0.00	0.20	0.00	-0.01	0.47
Ear height	0.03	-0.29	<u>0.95</u>	0.03	0.01	0.11	-0.15	-0.59	0.02	0.25	0.00	-0.02	0.35
Number of plants	0.01	-0.02	0.10	<u>0.34***</u>	0.00	0.02	-0.02	-0.06	0.06	-0.02	0.00	0.01	0.42
Grain moisture	-0.01	-0.02	0.15	0.00	<u>0.07</u>	0.03	-0.05	-0.11	0.05	0.10	-0.01	0.01	0.20
Days to anthesis	-0.04	-0.06	0.45	0.03	0.01	<u>0.25</u>	-0.38	-0.33	0.01	0.13	-0.01	-0.02	0.03
Days to silking	-0.05	-0.04	0.41	0.01	0.01	0.24	<u>-0.39</u>	-0.32	0.01	0.12	-0.02	-0.02	-0.03
Ear position	-0.03	-0.14	0.93	0.03	0.01	0.13	-0.19	<u>-0.67</u>	0.03	0.22	0.00	-0.02	0.31
Shelling percentage	-0.01	0.00	0.08	0.07	0.01	0.01	-0.01	-0.07	<u>0.28***</u>	0.04	0.00	0.01	0.40
Ear prolificacy	0.01	-0.17	0.59	-0.01	0.02	0.07	-0.11	-0.33	0.02	<u>0.45***</u>	0.00	-0.02	0.52
Root lodging	0.00	0.00	0.02	0.00	0.01	0.05	-0.09	-0.02	0.01	0.03	<u>-0.06</u>	0.00	-0.07
Stem lodging	-0.08	-0.03	0.22	-0.04	-0.01	0.07	-0.11	-0.15	-0.03	0.09	0.00	<u>-0.08</u>	-0.15

*, ** and *** indicate level of significance at 5%, 1% and 0.01% respectively.

Table 3.9: Direct (underlined and bold) and indirect effects of different traits in popcorn at Cedara (n= 120; R² =0.76)

Grain yields components	Ear length	Plant height	Ear height	Number of plants	Grain moisture	Days to anthesis	Days to silking	Ear position	Shelling percentage	Ear prolificacy	Root lodging	Stem lodging	Total correlation to grain yield
Ear length	<u>0.23***</u>	-0.04	0.07	0.01	0.09	-0.01	0.00	0.00	-0.03	-0.01	-0.01	0.04	0.34
Plant height	0.08	<u>-0.12</u>	0.22	0.04	0.15	0.01	-0.01	0.00	-0.04	0.17	-0.01	0.04	0.53
Ear height	0.06	-0.10	<u>0.27</u>	0.00	0.14	0.00	0.00	0.00	-0.02	0.21	-0.02	0.03	0.56
Number of plants	0.00	-0.01	0.00	<u>0.41***</u>	0.03	0.01	-0.01	0.00	0.02	-0.11	0.00	0.00	0.34
Grain moisture	0.07	-0.06	0.12	0.04	<u>0.30***</u>	0.00	0.00	0.00	-0.04	0.07	-0.02	0.06	0.55
Days to anthesis	0.01	0.01	0.00	-0.04	0.00	<u>-0.13</u>	0.09	0.00	0.00	0.02	0.00	0.00	-0.04
Days to silking	0.00	0.01	0.00	-0.05	-0.01	-0.12	<u>0.09</u>	0.00	0.00	0.02	0.00	0.00	-0.05
Ear position	0.01	-0.04	0.23	-0.04	0.08	-0.01	0.01	<u>0.00</u>	-0.01	0.18	-0.02	0.00	0.38
Shelling percentage	-0.04	0.03	-0.04	0.04	-0.07	0.00	0.00	0.00	<u>0.16**</u>	-0.02	0.00	-0.02	0.04
Ear prolificacy	0.00	-0.04	0.12	-0.09	0.05	0.00	0.00	0.00	-0.01	<u>0.48***</u>	0.00	0.02	0.51
Root lodging	0.03	-0.02	0.06	0.00	0.08	-0.01	0.01	0.00	0.00	0.00	<u>-0.07</u>	0.03	0.10
Stem lodging	-0.06	0.04	-0.05	0.00	-0.14	0.00	0.00	0.00	0.03	-0.06	0.02	<u>-0.13*</u>	-0.37

*, ** and *** indicate level of significance at 5%, 1% and 0.01% respectively.

3.3 Discussion

3.3.1 The frequency distribution of hybrids for yield and secondary traits

Grain yield at both Cedara and Ukulinga was skewed to the right implying that most hybrids were low yielding. The grain yield for Cedara ranged from 0 to 6.5 t ha⁻¹ and the average yield was 2 t ha⁻¹. Grain yield at Ukulinga ranged from 0 to 6 t ha⁻¹ and the average yield was 3 t ha⁻¹. This could be attributed to low rainfall during the critical growth stage (flowering) therefore the majority of the hybrids were not able to produce high yields. The distribution was continuous for grain yield which thus emphasize that it was controlled by many genes. These results are consistent with Holland (2007) who reported that genetic variation for complex traits such as yield is controlled by many genetic factors with relatively small effects. Many hybrids had low ear prolificacy. This might be due to the fact that growth assimilates were more focused on plant growth and the upper ear which enjoys apical dominance.

3.3.2 Mid-parent heterosis

Considerable amounts of heterosis were observed for all the traits of popcorn hybrids under study, and the magnitude varied with traits. All of the top 10 hybrids showed positive heterosis (292.68% to 410.61%) for grain yield, indicating existence of substantial heterosis in the hybrids with implications for breeding. These results are consistent with other reports by Wegary et al. (2013) and Legesse et al. (2008). The level of mean Mid-Parent Heterosis (MPH) (342.43%) for grain yield observed in the current study is higher than that reported by Wegary et al. (2013) and Saleh et al. (2002). This could be due to difference in genotypes used and the environment the hybrids were grown. MPH estimates varied considerably from trait to trait; generally highest estimates were obtained mostly for yield related traits such as ear length, plant height and ear height. The range of MPH estimates for plant height was positive for the top 10 hybrids. These results are in line with Iqbal et al. (2010) who reported positive heterosis for plant height and also Uzrowska et al. (2007) who observed substantial MPH for plant height ranging from 38 to 56% but were lower than those reported by Saleh et al. (2002). The range of MPH for ear height was positive for the top 10 hybrids. This is in contrast with Ali et al. (2012) who reported MPH for ear height ranging from -35 to 86% and plant height ranged from -18 to 75%. Heterosis in negative direction is desirable for ear height but in the current study no hybrid showed negative heterosis for the top 10 hybrids. MPH for

days to silking was negative for the top 10 hybrids and these results are in line with Iqbal et al. (2010) who reported negative MPH for mid-silk and Saleh et al. (2002) indicating that hybrids would flower ahead of the parents. Negative heterosis for days to silking and days to anthesis indicated possibilities for breeding of popcorns for earliness. Estimates for shelling percentage were positive and these are in line with Saleh et al. (2002). Overall superiority of the popcorn hybrids over their parents was confirmed. Therefore, a hybrid oriented breeding strategy will be pursued.

3.3.3 Relationship among phenotypic traits

The study showed that there were significant correlations among phenotypic traits at both sites. Grain yield exhibited highly significant ($p < 0.01$) and positive association with plant height, ear length, grain moisture, ear position and shelling percentage. This implied that increasing expression of these traits could positively influence grain yield potential. Furthermore, the positive relationship indicated that favourable genes controlling these traits could be utilized for improvement of these lines in future breeding programs. In addition, grain yield was highly significantly ($p < 0.01$) correlated with plant height and ear height at both sites. These results are consistent with Abirami et al. (2007) who found that plant height, ear height and ear length showed significant positive association with grain yield. Grain yield was strongly correlated with number of plants at both sites. This is expected as the more the ears the higher the chances of high yields. There was a strong correlation between anthesis date and silking date at both sites. This means that selecting for anthesis date will change the silking date by a similar magnitude. These results are in agreement with Olakojo and Olaoye (2011) who found a strong correlation (0.74) between anthesis date and silking date.

3.3.4 Path coefficient analysis

The dependent variable grain yield is mostly influenced by the direct effect of an independent variable and its indirect effects via other yield components. In the present study, path analysis was used to work out the direct and indirect effects of 12 traits on grain yield. Path coefficient analysis indicated that ear height showed positive direct effects on grain yield which is in agreement with Sreckov et al. (2011) and Akbar et al. (2008). Plant height showed negative

direct effects on yield and these results are consistent with Zarei et al. (2012) who reported that plant height had negative direct effects on yield. However, the findings are in contrast with Abirami et al. (2007) who reported direct and positive effects of plant height on grain yield. This may be attributed to the different genotypes used in this study. Ear length had higher positive direct effects on grain yield, which is in agreement with Hepziba et al. (2013) who reported high positive direct effects of ear length on grain yield. Days to silking showed higher negative direct effects on grain yield. This is consistent with Ojo et al. (2006). Raghu et al. (2011) reported direct negative effects by days to 50% silking. Shelling percentage had positive direct effects on grain yield, which is in agreement with the findings of Ojo et al. (2006). Positive direct effects of shelling percentage on grain yield suggest that popcorn hybrids with high grain yield can be obtained through selection for high shelling percentage. Therefore, breeders should give special attention to these traits. Though plant height had high positive and significant association with grain yield, its direct effects were negative, meaning direct selection for plant height may not necessarily improve yield. Indirect effects were ranked as described in Chapter 2. Root lodging was negligible at both sites. Plant height, days to silking and ear position had high indirect effects at Ukulinga. While at Cedara, plant height and days to silking exhibited low indirect effects on grain yield.

3.4 Conclusion

The objectives of the study were to determine heterosis in popcorn experimental hybrids and to estimate direct and indirect effects of yield component traits on grain yield. From this it can be concluded that,

- The top 10 hybrids with 292.68 % to 410.61% heterosis can be utilized in heterosis breeding to exploit hybrid vigour. The desirable heterosis observed in this study for grain yield and earliness indicated the potential for hybrid development. All estimates for days to anthesis and days to silking were negative; this clearly indicates that hybrids flowered earlier than their respective inbred parents.
- From the study it was observed that most of the traits were associated with grain yield. This indicated that selection for any one of these yield attributing traits will lead to increase in the other traits, and finally boosting grain yield. Hence, primary selection for traits like ear height and ear length will be given priority and higher value in this

selection since this will increase the number of grains per ear and hundred seed weight and finally will result in increased grain yield.

- The main significant direct factors contributing to yield were ear prolificacy, number of plants, ear length and shelling percentage indicating that direct selection for these traits would be effective to improve grain yield of hybrids. These traits can be considered as important quantitative traits in a popcorn improvement programme and direct selection for these traits would be rewarding.
- Most indirect effects of secondary traits on yield were high, this implies that indirect selection of these traits would improve yield potential of hybrids

Overall, the results will be crucial for devising a breeding strategy for popcorn hybrids.

3.5 References

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Diversity and Path Coefficient Analysis of Southern African Maize Hybrids

Abstract

Detailed knowledge about genetic diversity among germplasm is important for breeding. The objectives of the study were therefore to determine genetic diversity in widely grown hybrids in Southern Africa, to determine the relationship between traits and to compare effectiveness of phenotypic analysis models for determining genetic distances between hybrids. For SNP study, leaf discs were harvested and sent to DNA landmarks for genotyping. For phenotypic analysis, fifty maize hybrids were evaluated at one site in two replications. The experiment was laid out as a randomised complete block design. Phenotypic and genotypic data were analysed using SAS and Power maker respectively. There was significant ($p < 0.01$) variation and diversity among hybrid brands but small within brand clusters. Polymorphic Information Content (PIC) ranged from 0.07 to 0.38 with an average of 0.34 and genetic distance ranged from 0.08 to 0.50 with an average of 0.43. SAH23 and SAH21 (0.48) and SAH33 and SAH3 (0.47) were the most distantly related hybrids. The lowest genetic distances were found between SAH6 and SAH24 (0.01), SAH34 and SAH25 (0.01), SAH34 and SAH37 (0.01) and SAH37 and SAH25 (0.01). Both SNP markers and phenotypic data models were effective for discriminating genotypes according to genetic distance. SNP markers revealed nine clusters of hybrids. The 12-trait phenotypic analysis model, revealed eight clusters at 85%, while the five-trait model revealed six clusters. Path analysis revealed significant direct and indirect effects of secondary traits on yield. The identified influential traits will be exploited to improve yield through direct and indirect selection approaches. Plant height and ear height were negatively correlated with grain yield. Ear weight, days to anthesis, number of ears and number of plants had highest positive direct effects on yield. These traits could provide good selection indices for high yielding maize hybrids. The results confirm previous findings that diversity of hybrids is small within brands and also confirm that phenotypic trait models are effective for discriminating hybrids.

Key words: Genetic diversity, grain yield, maize, phenotypic traits

4 Introduction

Maize is one of the principal staple foods in Southern Africa. However, adequate production of maize is hampered by poor yields (M'mboyi et al., 2010). There is need therefore to improve yield of hybrids through breeding. Information on genetic diversity in maize is of fundamental importance in crop improvement (Choukan, 2011). It helps in classifying germplasm in appropriate heterotic groups for future breeding programs because genetically divergent genotypes are known to have high hybrid vigour (Dandolin et al., 2008). Knowledge on the genetic diversity is also important to plant breeders so that they know the extent of already existing genetic variability in the material and improve on them (Cholastova et al., 2011). There is much emphasis on the use of diverse genotypes because they contribute to high yields. Hallauer and Miranda. (1988) reported that divergent population improvements usually targets to increase yield and heterosis.

Genetic distance can be estimated by using a number of methods and these include pedigree data and molecular markers (Semagn et al., 2012). Various types of molecular markers can be used to estimate GD and these include RFLP, RAPD, AFLP, SSR and SNP markers. Nowadays there is a shift towards the use of SNP markers, because of their low cost per data point, high genomic abundance, co-dominance, potential for high throughput analysis and lower genotyping error rates (Foster et al., 2010; Semagn et al., 2012). Semagn et al.(2012) reported that SNP markers have emerged as a powerful tool for many genetic applications in genetic diversity studies and marker assisted breeding. Lu et al. (2011) compared the genetic diversity using SNP markers between tropical and temperate lines and found out that tropical germplasm had substantial higher genetic diversity 0.238-0.548 than temperate 0.224-0.473. Semagn et al. (2012) evaluated genetic distance for elite lines using SNP markers and results showed that 94% of the pair lines fell between 0.300-0.400. Therefore in this study SNP markers were used. Diversity can be also studied using phenotypic traits. Hepziba et al. (2013) studied diversity using phenotypic traits and reported that phenotypic traits were very effective as they clustered 70 maize inbred lines into 10 clusters. Similar studies were also conducted by Wu et al.(2012).

Cheres et al. (2000) reported that estimation of GD with the use of phenotypic characteristics gives less accurate results. This was in contrast with Bertan et al. (2007) who reported that phenotypic traits were very effective in estimating genetic distance. However, Leal et al. (2010) reported that molecular markers have proved to have advantages over other methods because they show genetic differences on a more detailed level without interferences from environmental factors and they involve techniques that provide fast results detailing genetic diversity. Therefore, one of the aim of this study was to evaluate the effectiveness of using both molecular markers and phenotypic traits in determining GD.

Genetic distance can be estimated using Mahalanobis (D^2) or Euclidean distance (Bertan et al., 2007). A clustering method is then used to separate the observations into many subgroups in order to obtain homogeneity within and between these groups (Darbeshwar, 2000; Bertan et al., 2007). Hierarchical methods are used to group genotypes thereby forming a dendrogram without concern for the number of groups formed. Different clustering methods are used depending on the procedure that is most suitable for the data set. Data obtained from the clusters can be used to make conclusions on the potential heterotic groups and new heterotic patterns which improve yield in maize hybrids. Hence, in this study the Euclidean distance was used to estimate genetic distance.

The objectives of this study were:

- (i) to determine genetic diversity in widely grown Southern African hybrids
- (ii) to determine the relationship between grain yield and secondary traits
- (iii) to compare effectiveness of phenotypic analysis models for determining genetic distances between maize hybrids
- (iv) to confirm efficacy of a small set of SNP markers less than 100 for discriminating genotypes in a diversity study.

4.1 Materials and methods

4.1.1 Germplasm

Fifty (50) commercial maize hybrids were selected for the genetic diversity study and were coded for convenience, as SAH1 to SAH50. The hybrids were drawn from nine brands that are available on the market in Southern Africa.

4.1.2 Genotyping

Single Nucleotide Polymorphism markers were used (Appendix 4.1). The 50 maize hybrids were grown in a tunnel with four pots per hybrid. Leaf tissues were harvested from each of the four plants at the four week stage. Two leaf discs (punches or equivalent) approximately 20 mm in diameter were harvested from each plant and were put in specific well positions. A sheet of Air Pore Tape was put on top of the block to seal. The block was then placed inside a plastic bag together with 50 g of silica gel and the materials were then dried for 24 hours. The indicator gel was used to confirm if it was dehydrated (blue when it is dehydrated or pink when hydrated). The samples were then shipped to the DNA landmarks laboratory in Canada for genotyping.

DNA was extracted following Sarkosyl Nitrogen method at the DNA landmarks. The method is described by Hasan et al. (2008). Freshly healthy leaves were placed in a mortar, frozen in liquid nitrogen and the material was crushed to fine powder using a pestle. 3ml of DNA extraction buffer was added to the powder and the mixture was homogenized. The mixture was then transferred to a test tube with a cap, 2ml of phenol was added and the mixture was centrifuged for 5 minutes to separate the phase. The upper aqueous phase was then transferred into a new tube. Two volumes of ice-cool 95% ethanol were added to the aqueous phase for ethanol precipitated DNA and centrifuged for 5 minutes at 12 000 rpm. The precipitated DNA was then washed with ice-cool 70% ethanol. The DNA was then dissolved in 0.5ml TE and 2µg RNase was added, the mixture was then incubated at 37°C for 15-30 minutes. 0.25ml of phenol and 0.25ml chloroform was then added, the mixture was shaken, centrifuged and the upper aqueous phase was transferred to a new tube. Two volumes of ice-cool 45% ethanol were then added to the aqueous phase for precipitated DNA and centrifuged for 5 minutes at 12 000 rpm. The precipitated DNA was then washed with ice-cool 70% ethanol and dissolved in 0.2 to 0.5 ml of TE.

Genotypic data was analysed using Power maker (version 3.25) statistical package. Polymorphic information content (PIC) values were calculated at each locus using the following formula:

$$PIC = 1 - \sum_{u=1}^k \tilde{p}_u^2 - \sum_{u=1}^{k-1} \sum_{v=u+1}^k 2\tilde{p}_u^2 \tilde{p}_v^2$$

PIC values give an estimate of the discriminatory power of a marker by taking into account not only the number of alleles at the locus but also the relative frequencies of these alleles.

Allelic diversity was calculated as follows:

$$Div = 1 - \sum_{u=1}^k \tilde{p}_u^2 \quad (\text{Botstein et al., 1980}).$$

4.1.3 Field trial design and management

The hybrids were evaluated at Ukulinga Research Station during the 2012/2013 season. The trial was planted on 26 November 2012. The experiment was laid out as a Randomised Complete Block Design with three replications. Each plot comprised of four rows of 5 m, 0.75 m between rows and 0.30 m within rows. The experiment was rain fed. The distribution of the monthly rainfall for the growing season is shown in Figure 2.2. The temperature data is shown in Figure 2.3. Fertilizer was applied as basal at planting in the form of a compound (NPK) 2:3:4 at 250 kg ha^{-1} (56 kg ha^{-1} of N, 83 kg ha^{-1} of P and 111 kg ha^{-1} of K). Nitrogen fertilizer was applied at 4 weeks after crop emergence in the form of LAN (Lime Ammonium Nitrate, 28%N) at the rate of 250 kg ha^{-1} . The herbicides, gramoxone, dual, basagran, and 2, 4-D were applied to control weeds. This was augmented by hand weeding to keep the fields relatively clean of weeds throughout the season. Carbaryl was applied in the maize leaf whorls for stalk borer control. An insecticide, karate, was applied to control cutworm at planting and seedling emergence.

4.1.4 Data collection

The following traits were measured as described in chapter 2; grain yield, plant height, ear height, number of plants, grain moisture, stem lodging, ear prolificacy, days to anthesis and

days to silking. In addition the following traits were measured following the standard protocols used at CIMMYT (Magorokosho et al., 2009):

a) **Ear weight:** measured as the weight of all the ears per plot

b) **Tillering:** measured as the number of primary branches.

4.1.5 Data analysis

The dendrogram for SNPs markers was constructed using Power marker (version 3.25) Liu. 2004). Phenotypic dendograms were constructed using GENSTAT (version 14th edition). General analysis of variance was performed using SAS 9.3 version (SAS Institute Inc, 2011). The hybrid data was analysed as Randomised Complete Block Design using the following model:

$$Y_{ij} = \mu + B_i + T_j + E_{ij}$$

Where, Y_{ij} = observed response,

μ = grand mean

B_i = effect of the i^{th} block, $i = 1 \dots 3$

T_j = effect of the j^{th} treatment, $j = 1 \dots 50$

E_{ij} = random experimental error

Path analysis and correlation analysis were performed in SAS version 9.3 (Scott and Milliken, 1993).

4.2 Results

4.2.1 Molecular Marker Characterization

There was considerable variation between hybrids. The characteristics of the 94 SNP markers analysed are shown in Figure 4.1 and Figure 4.2. Only three markers (PZB02480_1, PZB02033_2 and PZB00175_6) had a low call rate of 0%, 5% and 85% respectively so they were not included in data analysis. The PIC ranged from 0.07 (PZA03728_1) to 0.38 (PZA01735 and PZA01755_1) with an average of 0.34. Approximately 65% of the markers used (61 out of 94) had PIC values exceeding 0.30, demonstrating the good discriminatory power of the markers suggesting that considerable variation between hybrids is detectable with SNP markers. SNP diversity data ranged from 0.08 to 0.50 with an average of 0.43, however, the vast majority (85.7%) fell between 0.30 and 0.50 (Figure 4.2).

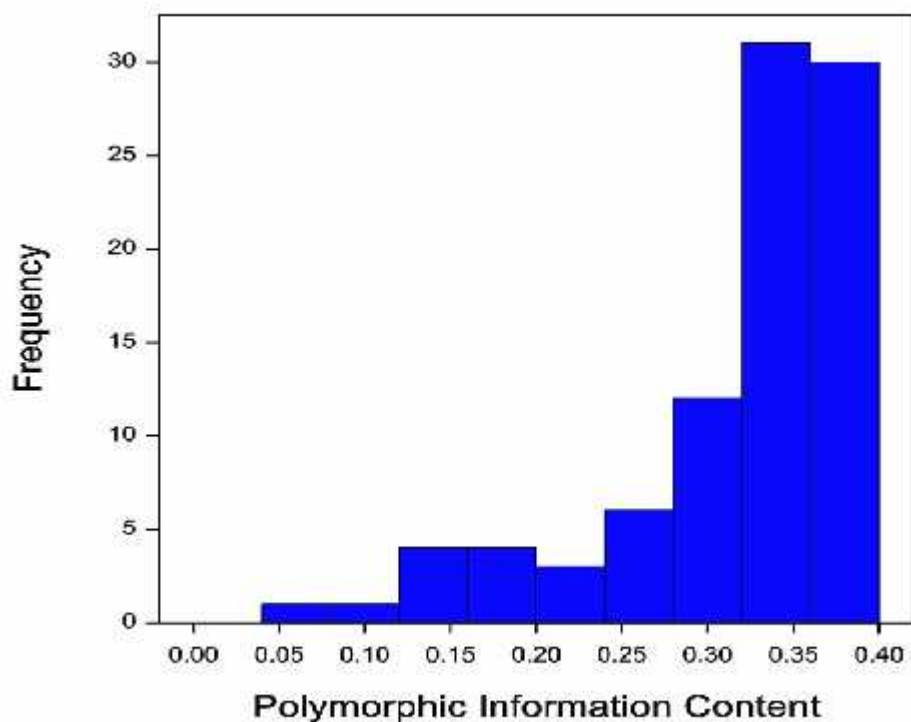


Figure 4.1: Distribution of the 91 SNP markers for Polymorphic Information Content

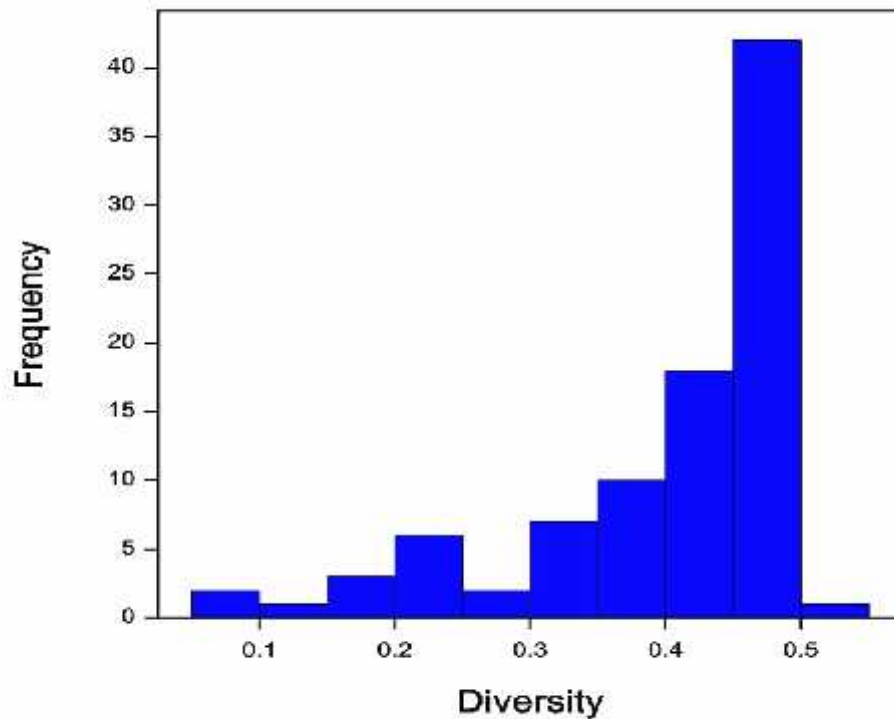


Figure 4.2: Distribution of the 91 SNP markers for diversity

4.2.2 Genetic distance among hybrids

There was a large diversity among the hybrids. Using SNP markers the highest genetic distance between the hybrids was 0.48 and the lowest was 0.01. The highest genetic distance; above 0.40 were found between SAH23 and SAH21, SAH33 and SAH1, SAH32 and SAH18 and SAH33 and SAH23. Conversely, the lowest genetic distance (0.01) was found between SAH6 and SAH24, SAH34 and SAH25, SAH34 and SAH37 and SAH37 and SAH25.

Using phenotypic traits, the highest genetic distances were found between SAH43 and SAH1 and SAH33 and SAH1. Conversely, the lowest genetic distances were found between SAH31 and SAH40, SAH5 and SAH42 and SAH41 and SAH38. Genetic distance summary for 50 hybrids measured against a tropical hybrid SAH33 are shown in Figure 4.3. Almost all hybrids fell between 0.25 and 0.48, with an exception of one hybrid that had a genetic distance which was lower than 0.1.

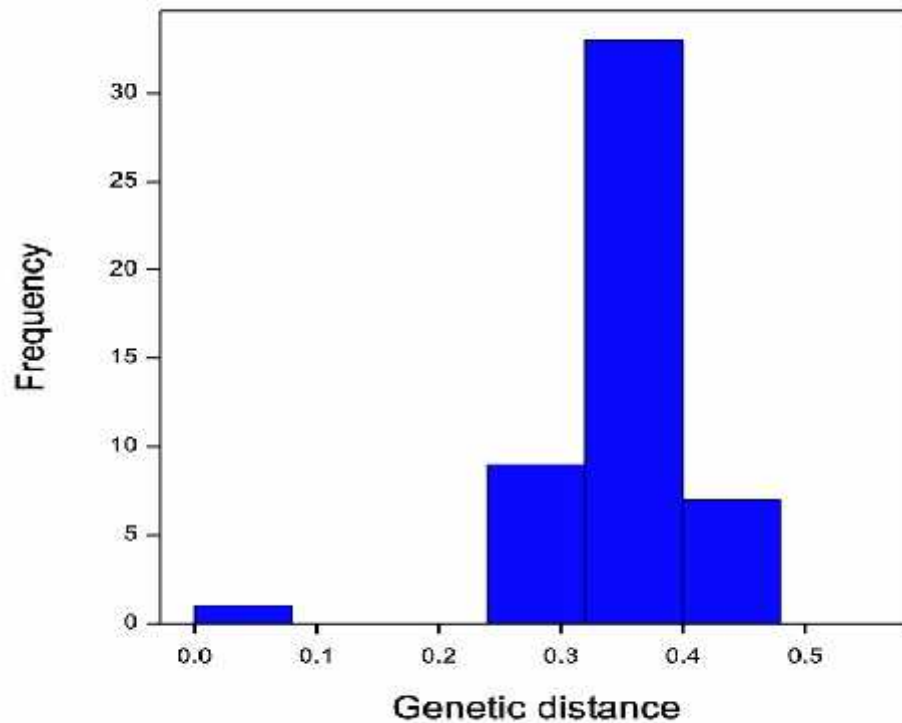


Figure 4.3: Genetic distance data summary of 50 hybrids using phenotypic traits measured against a tropical hybrid (SAH33)

4.2.3 Cluster analysis of hybrids based on molecular markers

SNP markers were effective for discriminating the hybrids into different clusters (Figure 4.4). The genotypes were grouped in two major clusters I and II, whereby cluster II was the largest cluster and cluster I had two hybrids only. Cluster II was further divided into five clusters (A, B, C, D, and E) in which cluster B was the largest with 30 hybrids followed by cluster C with 11 hybrids, cluster D with 4 hybrids, cluster E with 4 hybrids and cluster A with 1 hybrid. Cluster B was further divided into three sub-clusters (B_1 , B_2 and B_3) and cluster C was divided into two sub-clusters (C_1 and C_2).

4.2.4 Cluster analysis based on 12 phenotypic traits model

Phenotypic data was effective for discriminating the hybrids into different clusters (Figure 4.5). The dendrogram of 50 maize hybrids was based on the following 12 traits: plant height, ear height, number of plants, number of ears, ear weight, grain weight, ear prolificacy, stem lodging, root lodging, tillers, pollen date and silking date. There were two major clusters I and II at a 0.6 similarity cut-off point; however four clusters (A, B, C and D) are shown at a cut-off point of 0.75. Furthermore, at 0.85 cut-off point, cluster B was sub-divided into B₁ and B₂, cluster C was sub-divided into C₁, C₂ and C₃ and lastly cluster D was sub-divided into D₁ and D₂.

4.2.5 Cluster analysis based on five phenotypic traits model

Phenotypic data using fewer traits (five) was less effective for discriminating the hybrids into different clusters compared when using more phenotypic data (Figure 4.6). The dendrogram of 50 maize hybrids was constructed using stem lodging, tillering, ear prolificacy, grain moisture and grain yield. There are two major clusters I and II at 0.60 cut-off point; however four clusters A, B, C and D are shown at a cut-off point of 0.75. Furthermore, at 0.85 cut-off point cluster A is sub-divided into A₁, cluster B into B₁ and cluster C into C₁ and C₂ and cluster D into D₁ and D₂.

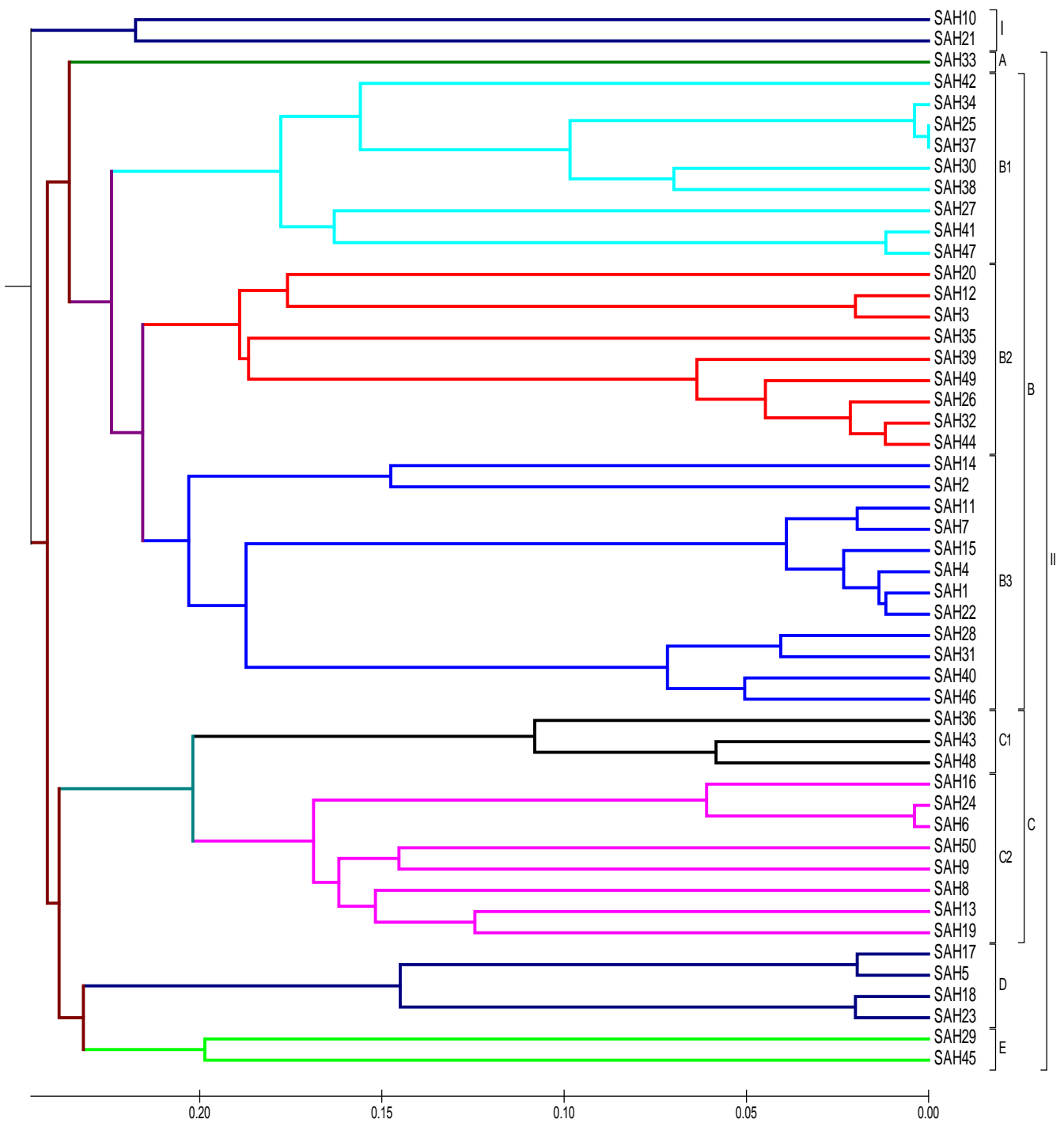


Figure 4.4: Dendrogram of 50 maize hybrids based on 91 SNP molecular markers using Euclidean distance.

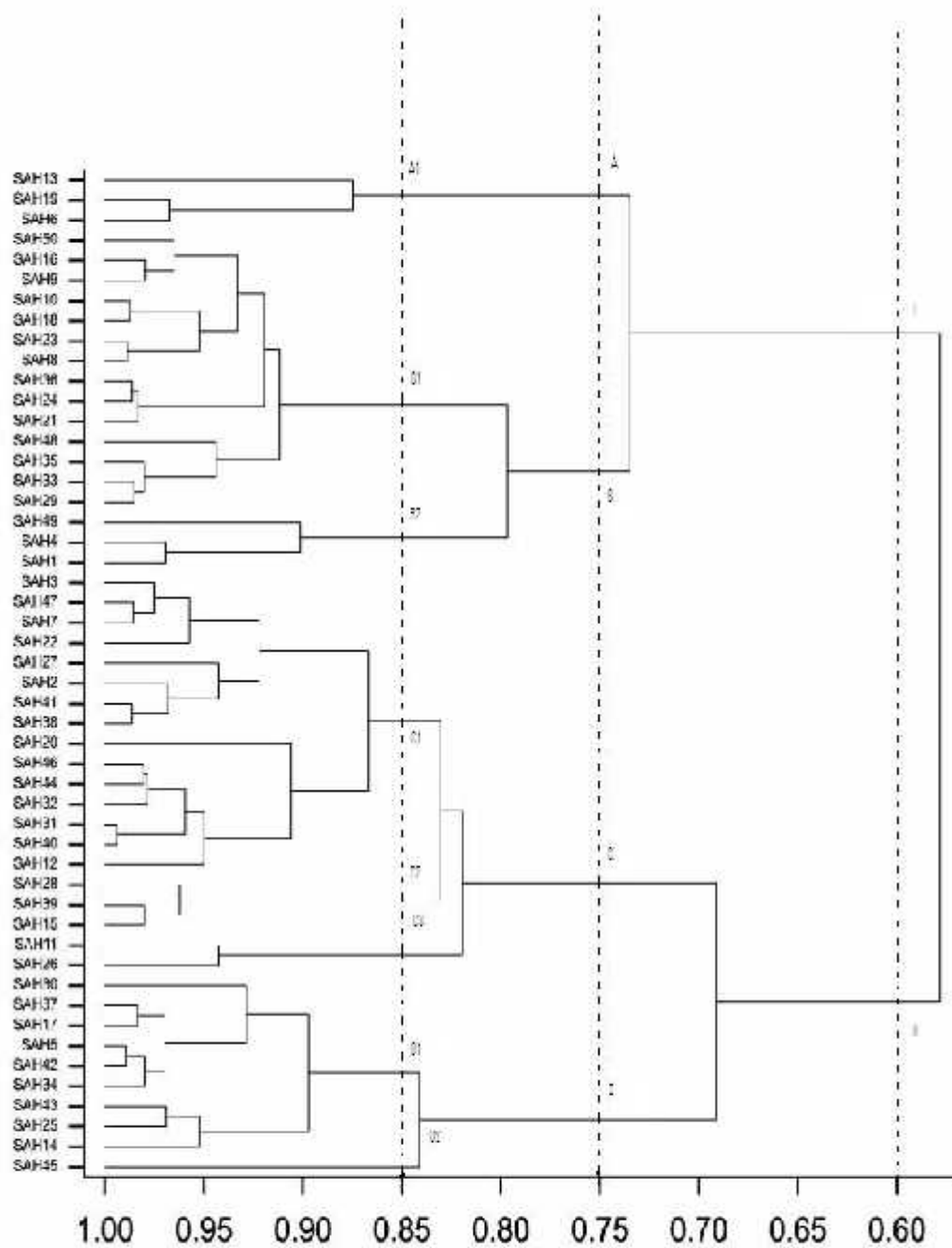


Figure 4.5: Dendrogram of 50 maize hybrids based on 12 phenotypic traits model using Euclidean distance.

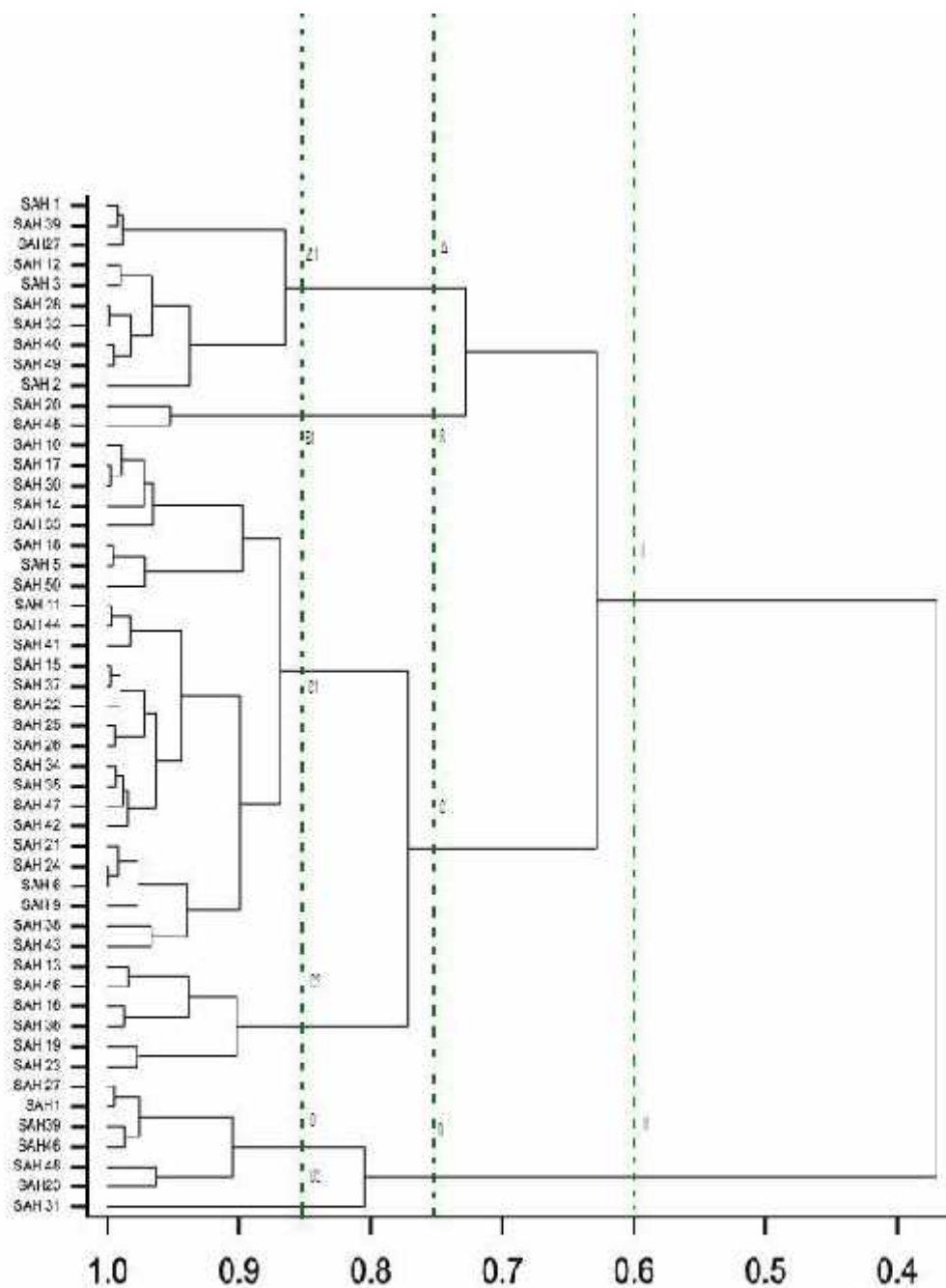


Figure 4.6: Dendrogram of 50 maize hybrids based on five phenotypic traits model using Euclidean distance.

4.2.6 Relationship between phenotypic traits

Differences were observed in correlation coefficients in terms of magnitude and direction. There was positive correlation among phenotypic traits though most were not significant (Table 4.1). Only ear prolificacy and ear weight showed positive correlations with grain yield (more than 0.50). However, plant height, ear height, days to anthesis and days to silking showed negative correlations with yield. Positive values of correlation coefficient were found among almost all other traits with a few exceptions of grain moisture and number of plants, tillering and ear prolificacy and tillering and ear weight. Plant height correlated significantly ($p < 0.01$) and positively with ear height, days to anthesis and days to silking (Table 4.1). Days to anthesis correlated significantly ($p < 0.01$) with days to silking, days to silking and grain moisture, days to anthesis and ear height. Ear prolificacy correlated significantly ($p < 0.05$) and negatively with number of plants.

Table 4.1: Relationship among phenotypic traits of 46 maize hybrids at Ukulinga

	Grain yield	Plant height	Ear height	Number of plants	Ear weight	Grain moisture	Stem lodging	Tillering	Ear prolificacy	Days to anthesis	Days to silking
Grain yield	-										
Plant height	-0.08	-									
Ear height	-0.06*	0.68**	-								
Number of plants	0.26	0.24	0.09	-							
Ear weight	0.64	0.20	0.09	0.64**	-						
Grain moisture	0.30	0.22	0.53*	0.17	-0.06	-					
Stem lodging	0.11*	0.10	0.27	0.17	0.10	0.32*	-				
Tillering	0.19**	0.22	0.23	-0.10	-0.11	-0.10	-0.26	-			
Ear prolificacy	0.51	0.02	0.18	0.21	0.08	0.20	0.29	0.14	-		
Days to anthesis	-0.08*	0.41*	0.63**	0.20	0.09	0.56**	0.34*	0.13	0.33*	-	
Days to silking	-0.05*	0.38*	0.61**	0.24	0.10	0.55**	0.35*	0.14	0.36*	0.99**	-

*, ** and*** indicates level of significance at 5% and 1%.

4.2.7 Path coefficient analysis

Direct effects of secondary traits were more influential on yield compared to indirect effects. Regression data showing the level of significance for direct effects is presented in Table 4.2. Ear weight and grain moisture were the only traits that were highly significant ($p < 0.0001$) for direct effects on yield. The dependent variable grain yield is mostly influenced by the direct effects of an independent variable and its indirect effects via other yield components. Ear weight exhibited the highest direct and positive effects on yield followed by days to anthesis, number of ears and ear height (Table 4.3). However, grain moisture displayed the highest negative direct effects on grain yield followed by days to silking, ear prolificacy and number of plants. Number of ears showed indirect positive effects on grain yield through ear weight followed by ear prolificacy via ear weight, grain moisture through ear weight and number of plants via ear weight. Highest indirect negative effects on grain yield were exhibited by ear weight through grain moisture, followed by days to anthesis via days to silking, number of ears through prolificacy and ear height via grain moisture.

Table 4.2: Regression data showing levels of significance for direct effects of secondary traits on yield in maize hybrids

Trait	Parameter Estimate	Standard error	t-value	P-value
Plant height	0.0042	0.0365	0.11	0.9100
Ear height	0.0095	0.0483	0.20	0.8450
Number of plants	-0.0476	0.0295	-1.61	0.1162
Number of ears	0.0410	0.0968	0.42	0.6750
Ear weight	1.1551	0.0430	26.88	<0.0001***
Grain moisture	-0.2629	0.0397	-6.62	<0.0001***
Days to anthesis	0.11619	0.3074	0.38	0.7079
Days to silking	-0.1334	0.3040	-0.44	0.6637
Ear prolificacy	0.0281	0.0563	0.50	0.6210
Stem lodging	-0.0487	0.0320	-1.52	0.1368
Tillering	-0.0159	0.0326	-0.49	0.6291

*, ** and*** indicates level of significance at 5%, 1% and 0.01%, respectively

Table 4.3: Direct (underlined and bold) and indirect effects of different traits in 46 maize hybrids at Ukulinga (n=46; R²=0.63)

Grain yield component	PH	EH	NP	NE	EW	GM	Ear prolificacy (EPP)*	DA	DS	SL	TL	Total correlation to grain yield
Plant height (PH)	<u>0.00</u>	0.01	-0.01	0.00	-0.06	-0.01	0.00	0.05	-0.05	0.00	0.00	-0.08
Ear height (EH)	0.00	<u>0.01</u>	-0.01	0.01	0.07	-0.10	-0.02	0.07	-0.08	-0.01	0.00	-0.06
Number of plants (NP)	0.00	0.00	<u>-0.05</u>	0.01	0.28	-0.03	-0.01	0.02	-0.02	0.01	0.00	0.20
Number of ears (NE)	0.00	0.00	-0.01	<u>0.04</u>	0.70	-0.09	-0.10	0.05	-0.06	-0.01	0.00	0.52
Ear weight (EW)	0.00	0.00	-0.01	0.02	<u>0.86***</u>	-0.14	-0.06	0.00	-0.01	-0.01	0.00	0.64
Grain moisture (GM)	0.00	0.00	-0.01	0.01	0.61	<u>-0.26***</u>	-0.03	0.03	-0.04	-0.01	0.00	0.31
Ear prolificacy (EPP)*	0.00	0.00	0.00	0.04	0.67	-0.07	<u>-0.10</u>	0.04	-0.05	-0.01	0.00	0.52
Days to anthesis (DA)	0.00	0.01	-0.01	0.02	0.03	-0.08	-0.03	<u>0.12</u>	-0.13	-0.02	0.00	-0.08
Days to silking (DS)	0.00	0.01	-0.01	0.02	0.08	-0.08	-0.04	0.12	<u>-0.13</u>	-0.02	0.00	-0.05
Stem lodging (SL)	0.00	0.00	0.01	0.01	0.23	-0.06	-0.03	0.04	-0.05	<u>-0.05</u>	0.00	0.11
Tillering (TL)	0.00	0.00	0.00	0.01	0.17	0.04	-0.01	0.02	-0.02	0.01	<u>-0.02</u>	0.19

*, ** and*** indicates level of significance at 5%, 1% and 0.01%, respectively

4.3 Discussion

4.3.1 Polymorphism of the SNP markers

Of the 94 SNPs identified 91, were used with only three being dropped because they had a low call rate (<90%). PIC had the highest value of 0.38 and these results are in line with Hao et al. (2011) who reported highest value of PIC of 0.375 using 1536 SNP markers on 95 maize inbred lines. These results are also in accordance with Yang et al. (2011) who reported the highest value of PIC of 0.38 using 884 SNP markers. The average PIC value in this study was higher, 0.34 compared to PIC of 0.239 reported by Hao et al. (2011) and Lu et al. (2009) who reported average PIC value of 0.25 using 1034 SNP markers on 770 maize lines. The efficacy of SNP markers used in the study can be explained by the fact that they were carefully selected at a ratio of 10 per chromosome and their uniform coverage of the genome. Therefore, the diversity data generated can be considered reliable. This set of SNP markers will be recommended for future studies for genotyping maize inbred lines.

4.3.2 Genetic distance

There was genetic variation between the maize hybrids. SAH23 and SAH21 (0.48) and SAH33 and SAH3 (0.47) were the most distantly related hybrids. This explained why these hybrids belonged to different genetic clusters. Hence they have the potential to produce superior hybrids when crossed. This is in line with Hallauer and Miranda (1988) who asserted that the genetic divergence of parental varieties determines the manifestation of heterosis, and that the heterotic patterns are determined by the genetic divergence of two parental varieties. The lowest genetic distances were found between SAH6 and SAH24 (0.01), SAH34 and SAH25 (0.01), SAH34 and SAH37 (0.01) and SAH37 and SAH25 (0.01) indicating that they were most closely related to each other. Therefore they belong to the same heterotic group and have less potential to produce superior hybrids when crossed. This is because crossing germplasm that is closely related results in inbreeding depression rather than hybrid vigour. The minimum genetic distance from the study confirmed the ability of SNP markers to distinguish closely related hybrids.

4.3.3 Cluster analysis based on molecular data and phenotypic data

SNP markers

The SNP markers were effective for discriminating maize hybrids according to genetic backgrounds and brands. Hybrids that were clustered in sub-cluster B₁ SAH 3 and SAH 12 are derived from the same brand. The same applies for SAH 25, SAH 34 and SAH 37. Hybrid lines in sub-cluster B₂ SAH 12, and SAH 3 are also from the same brand. In addition, SAH 6 and SAH 24 which were in sub-cluster C₂ have the same background. Similarly, SAH 5 and SAH 17 which were in cluster D, are derived from the same brand. Similar results have been reported in studies conducted by Yan et al. (2010) who reported seven clusters obtained by using 1362 SNP markers. Schaefer and Bernardo (2013) reported the effectiveness of SNP markers as they managed to group 284 maize inbreds into five clusters. Yang et al. (2011) reported that the same clusters were observed by using both SSR and SNP markers.

Cluster analysis based on phenotypic traits

Phenotypic trait models were effective in discriminating maize hybrids. Most of the hybrids in cluster A were non-prolific, had low ear placement, same number of plants and early maturing. SAH13 was then found alone because it had 25% stem lodging and 11% root lodging. This can be due to the fact that SAH13 is not adapted to the South African environment. It is a tropical hybrid. Hybrids in cluster B had medium plant height and medium ear placement. Most of the hybrids had the highest number of plants, moderate prolific and late maturing. Three hybrids SAH1, SAH4 and SAH49 were in sub-cluster B₂ because they had the highest number of ears compared to the rest of the hybrids. Further, hybrids in cluster C were taller, high prolific, high ear placement and moderate number of plants. Most hybrids in cluster C had high stem lodged plants. Furthermore, hybrids in cluster C₂ had the same number of plants and also the same number of plants that were prolific. They also had higher number of plants with stem lodging compared to the other clusters. Hybrids in cluster C₃ exhibited very high numbers of plants, number of ears, prolificacy and they had no plants with stem lodging and root lodging. These results are also supported by Shahrokhi and Khavarikhorasani (2013) who reported on the effectiveness of phenotypic traits as they

grouped 28 maize hybrids into four clusters. Furthermore, Karanja et al. (2009) reported that both phenotypic traits and SSR markers were effective in clustering inbred lines. Pedigree analysis placed 119 inbred maize lines into eight and SSR markers resulted in clustering the inbred lines into 10 groups (Reid et al., 2011). Similar results were reported by Wen et al. (2011) where nine main subsets were clustered using pedigree information.

4.3.4 Correlation among phenotypic traits

There was significant correlation among phenotypic traits. Grain yield exhibited negative correlation with plant height and ear height though for ear height it was significant ($p < 0.05$). These results are in line with Sreckov et al. (2011) but in contrast with Akbar et al. (2008) and Bocanski et al. (2009). This means that shorter plants and those with a low ear height produced higher yields. Grain yield showed highly significant ($p < 0.01$) negative association with days to anthesis and days to silking. These results showed that breeding for earliness has a potential of increasing yield unlike selection for flowering. Since this trait is less influenced by the environment, and is more influenced by genetic factors, it is more heritable and suitable when selecting for grain yield indirectly. These results are consistent with Pavan et al. (2011) who observed a negative correlation between grain yield and days to 50% silking but in contrast with Selvaraj and Nagarajan (2011) who reported that anthesis date and silking date showed positive non-significant association with grain yield.

The direct negative effects of days to silking on yield should be taken to imply that selection of early silking will result in selection for low yield potential. Grain yield showed highly significant ($p < 0.01$) and negative association with stem lodging. This means that if the plant is lodged its ability to transmit nutrients to the rest of the plant is reduced. This may be attributed to environmental factors such as heavy rains coinciding with wind or management factors such as high plant densities. It can also be due to the fact that the stems were more prone to pest and diseases hence stem lodging occurred.

4.3.5 Path coefficient analysis

Partitioning yield components into direct and indirect effects is of importance to the breeder so that traits that directly contribute to yield are selected. The results showed that ear weight had the highest significant direct effect on grain yield. Therefore, grain yield can be improved by selecting for ear weight. In this study, stem lodging had positive and significant correlation with grain yield, but when it was further partitioned using path analysis it had negative direct effects on grain yield meaning that direct selection for this trait can compromise yield of hybrids. Direct negative effects that were attributed to days to silking indicated that selection for late silk emergence results in less grain yield. This is in concurrence with earlier reports by Raghu et al. (2011). Ear weight had the highest direct and positive effects on grain yield, which is in line with Ilker (2011) who reported that maximum positive contribution of fresh grain yield was through ear weight. This indicates that this trait could be used more confidently as the selection criteria in the grain yield improvement of maize hybrids. Plant height had no direct contribution to yield, which is contrary to previous studies by Hepziba et al. (2013) who reported that plant height had direct and positive effects on grain yield.

The indirect effects were categorised into three groups where number of plants, stem lodging and tillering had negligible indirect effects on grain yield. Days to silking and ear prolificacy had low indirect effects and only grain moisture had moderate indirect effects on grain yield.

4.4 Conclusion

The objectives of the study were to determine genetic diversity in widely grown selected hybrids in Southern Africa, to determine the relationship between traits and to compare effectiveness of phenotypic analysis models for determining genetic distances between hybrids. Therefore it can be concluded that,

- There is large genetic diversity among widely grown maize hybrid brands in Southern Africa. However there is very little diversity within the brands suggesting that narrow genetic based populations have been used to derive the inbred parents for the hybrids. This is consistent with observations in other countries such as the USA.

- Both molecular and phenotypic data were effective for discriminating the hybrids into different clusters according to genetic background. SNP markers showed that there were nine clusters of hybrids. For the phenotypic data the level of discrimination of genotypes depended on the number of traits that were included in the model. With 12 trait model, phenotypic analysis revealed 8 clusters at 85%, while the five-trait model revealed six clusters that were substantially fewer than the nine clusters obtained from the SNP data. Using phenotypic traits, it was shown that discrimination capacity increased with an increase in number of traits, since further refinement was more powerful with 12 traits than five traits.
- Positive significant direct effects of ear weight on grain yield can be attributed to greater photosynthate which could have accumulated in each grain hence giving higher ear diameter. Consequently, this could be part of a good selection index for high yielding maize hybrids.
- Indirect effects played a minor role on determining yield potential of the hybrids because they were generally low, moderate or negligible.

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General Overview of the Study and Future Directions

5 Introduction

The yield plateau in maize is limited due to limited variety technology options for farmers. Grain yield is generally low yet food requirements have increased tremendously leading to a huge gap between population growth and food production in sub-Saharan Africa. This is mainly attributable to poor genetic improvements in maize and equally poor management practices. In SSA maize is extensively grown for consumption and generating income for farmers. Global demand of maize is estimated at 852 million tonnes by 2020.

Popcorn in sub-Sahara Africa is hampered by non-availability of superior varieties with high grain yield potential. This is associated with limitations on information on varieties with desirable agronomic traits. There is not even a single popcorn hybrid that has been produced in South Africa since 1954 which is adapted to local conditions hence farmers have been relying on imported hybrids. Therefore, there is need to develop new heterotic patterns that have hybrid vigour and popcorn hybrids that give high yield potential at the same time adapted to the local conditions.

The specific objectives of the study were:

1. To determine standard heterosis, the levels of variation and heritability for phenotypic traits in dent and flint maize hybrids
2. To determine the association between genetic distances and phenotypic traits in dent and flint maize hybrids
3. To determine mid-parent heterosis in popcorn hybrids
4. To determine the effect of secondary traits on grain yield in dent, flint and popcorn hybrids
5. To determine genetic diversity and the relationship between traits in widely grown selected hybrids in Southern Africa

6. To compare effectiveness of phenotypic analysis models for determining genetic distances between hybrids.

5.1 Findings from the literature

The literature reviewed indicated that:

- There is limited research that has been done on heterosis in popcorn. Similar amounts of resources have not been put on popcorn because it is regarded as a snack compared to dent and flint maize.
- A few papers have reported on the use of both molecular markers and phenotypic traits to discriminate hybrids into different clusters.
- There is no reported literature regarding the levels of diversity in Southern Africa, which has implications on the maize breeding because of climate changes and other current environmental challenges.

5.2 Findings from the research completed

5.2.1 Genetic variation, heritability, heterotic patterns and genetic distance

- The study revealed that the association between yield and genetic distance depended on the environment because grain yield of hybrids correlated positively with genetic distance at one site but there was no correlation on the other site.
- There was significant GEI among the maize hybrids as hybrids that were in the top 10 at one site were different at the other site. However there was an exception of hybrid 181 which was in the top 10 at both sites.
- There were hybrids that were in the top 10 but they had negative heterosis relative to the mean of control hybrids and the better control hybrid, indicating that these hybrids were inferior to the control hybrids. Thus for advancement in the breeding program, one would recommend selection of hybrids 150, 162 and 179 at Ukulinga which were more superior to the standard checks at Ukulinga. All the hybrids in the top 10 at Cedara can be selected because they performed better than the control hybrids.

- The lines DXL124 and DXL158 dominated parentage of the top 10 hybrid rank for yield qualifying them as potential testers for specific combining ability in future studies.
- New heterotic patterns that performed better than the control hybrids can be utilized in heterosis breeding to enhance yield potential of maize hybrids. However, there is need to test them in many different environments to check on their stability because GEI was significant.
- Grain texture cannot be used to discriminate hybrids for yield because all patterns of dent x dent, dent x flint and flint x flint were present in the top 10 hybrids at both sites.
- The main direct factors contributing to yield were ear prolificacy, number of plants, ear height, cob length and shelling percentage. Therefore, these traits can be used as primary selection criteria for grain yield in the respective environments. However, indirect effects of plant height, plant aspect, ear height, and grain texture would also be exploited to improve yield via ear height and ear position.

5.2.2 Heterosis and path coefficient analysis in popcorns

There was considerable amount of heterosis observed in the study:

- The top 10 hybrids with 292.68% to 410.61% heterosis for yield would be advanced and used in future studies.
- Most of the traits in the study including plant height, ear height and ear prolificacy associated positively with grain yield and selection for these traits will boost yield.
- Main direct factors that were significant include ear length, number of plants, shelling percentage and ear prolificacy can be used as primary selection criteria to effectively improve yield of popcorn

5.2.3 Diversity

There is significant diversity among the South African maize hybrids:

- There was genetic diversity among the Southern African maize hybrid brands, however, there was little variation within brands

- On the basis of cluster analysis using SNP markers, the 50 maize hybrids were classified into two major groups and further divided into five sub-groups and the results showed that hybrids that were in the same group belong to the same brand
- Discriminating hybrids into different clusters using phenotypic traits was effective and it was shown that when using phenotypic traits, discrimination capacity increases with an increase in number of traits. 12-trait model was more powerful compared to the five-trait model.
- Main direct effects of secondary traits contributing to yield can provide a good selection index for high yielding maize, mainly ear weight.

5.3 General implications and the way forward

The following implications and future directions were identified:

- It is quite essential to further evaluate all the hybrids that perform better than the mean control and better control and to determine their stability across different environments in South Africa.
- There was significant GEI, indicating the need to evaluate hybrids in different locations and years
- The study revealed high genetic diversity in the maize hybrids, implying that there is a high potential for producing new and superior hybrids.
- Popcorn hybrids that performed better than the mid-parent can be utilized in heterosis breeding to exploit hybrid vigour, but there is need to test them across a wide range of different environments, in order to have hybrids that are adapted to the local conditions.

5.4 Conclusion and recommendation

The main objective of the study was to identify new heterotic patterns and diversity among dent, flint and popcorn hybrids in order to improve grain yield in maize hybrids. It is recommended that heterosis can be studied in maize hybrids from the same and different heterotic patterns. The results from the study revealed high genetic diversity in hybrids,

implying there is high potential for production of new and superior hybrids. There was GEI indicating the need to evaluate genotypes in different locations for a number of years to test on their stability. The traits with significant direct factors contributing to yield can be used as primary selection criteria for grain yield. Integration can be applied by using both molecular markers and phenotypic traits to study diversity in maize hybrids.

6 Appendix 4.1 SNP markers used in the study

Marker name	Allele	Allele count	Frequency	Diversity	PIC	Call rate
bt2_2	C	90	0.75	0.38	0.3	
bt2_2	T	30	0.25			100%
csu1171_2	G	81	0.68	0.44	0.34	
csu1171_2	A	39	0.33			100%
Fea2_1	G	79	0.66	0.45	0.35	
Fea2_1	C	41	0.34			100%
PHM4348_16	C	77	0.64	0.46	0.35	
PHM4348_16	T	43	0.36			100%
PZA00106_10	C	19	0.16	0.27	0.23	
PZA00106_10	G	101	0.84			100%
PZA00136_2	C	10	0.08	0.15	0.14	
PZA00136_2	T	110	0.92			100%
PZA00223_2	T	79	0.66	0.45	0.35	
PZA00223_2	C	41	0.34			100%
PZA00257_22	T	114	0.95	0.1	0.09	
PZA00257_22	C	6	0.05			100%
PZA00266_7	C	41	0.34	0.45	0.35	
PZA00266_7	T	79	0.66			100%
PZA00309_2	C	78	0.65	0.46	0.35	
PZA00309_2	A	42	0.35			100%
PZA00343_31	G	94	0.78	0.34	0.28	
PZA00343_31	A	26	0.22			100%
PZA00352_23	A	71	0.59	0.48	0.37	
PZA00352_23	G	49	0.41			100%
PZA00455_16	C	61	0.54	0.5	0.37	
PZA00455_16	A	53	0.46			95%
PZA00543_12	C	55	0.47	0.5	0.37	
PZA00543_12	A	63	0.53			98%
PZA00726_8	T	14	0.12	0.21	0.19	
PZA00726_8	C	104	0.88			98%
PZA00827_1	C	77	0.64	0.46	0.35	
PZA00827_1	A	43	0.36			100%
PZA00878_2	G	54	0.45	0.5	0.37	
PZA00878_2	C	66	0.55			100%
PZA00881_1	C	70	0.58	0.49	0.37	
PZA00881_1	A	50	0.42			100%
PZA00920_1	A	52	0.46	0.5	0.37	
PZA00920_1	G	62	0.54			95%
PZA00947_1	G	86	0.72	0.41	0.32	
PZA00947_1	T	34	0.28			100%
PZA00948_1	A	70	0.58	0.49	0.37	
PZA00948_1	T	50	0.42			100%
PZA01142_4	C	94	0.8	0.32	0.27	
PZA01142_4	T	24	0.2			98%
PZA01292_1	C	48	0.4	0.48	0.36	
PZA01292_1	T	72	0.6			100%
PZA01304_1	G	78	0.65	0.46	0.35	
PZA01304_1	A	42	0.35			100%
PZA01315_1	T	64	0.53	0.5	0.37	
PZA01315_1	C	56	0.47			100%
PZA01342_2	T	27	0.23	0.35	0.29	
PZA01342_2	C	91	0.77			98%
PZA01396_1	A	38	0.32	0.43	0.34	
PZA01396_1	G	82	0.68			100%
PZA01447_1	G	30	0.25	0.38	0.3	
PZA01447_1	A	90	0.75			100%
PZA01735_1	A	59	0.49	0.5	0.37	
PZA01735_1	G	61	0.51			100%
PZA01755_1	T	61	0.51	0.5	0.37	
PZA01755_1	A	59	0.49			100%
PZA01804_1	A	92	0.77	0.36	0.29	
PZA01804_1	G	28	0.23			100%
PZA02019_1	A	92	0.77	0.36	0.29	
PZA02019_1	G	28	0.23			100%
PZA02027_1	C	74	0.63	0.47	0.36	
PZA02027_1	T	44	0.37			98%
PZA02068_1	G	14	0.12	0.21	0.18	
PZA02068_1	A	106	0.88			100%

Marker name	Allele	Allele count	Frequency	Diversity	PIC	Call rate
PZA02113_1	C	81	0.69	0.43	0.34	
PZA02113_1	G	37	0.31			98%
PZA02148_1	G	52	0.45	0.49	0.37	
PZA02148_1	A	64	0.55			97%
PZA02212_1	G	63	0.53	0.5	0.37	
PZA02212_1	A	57	0.48			100%
PZA02367_1	T	106	0.88	0.21	0.18	
PZA02367_1	C	14	0.12			100%
PZA02386_2	A	107	0.91	0.17	0.15	
PZA02386_2	G	11	0.09			98%
PZA02450_1	G	51	0.43	0.49	0.37	
PZA02450_1	T	69	0.58			100%
PZA02564_2	T	87	0.73	0.4	0.32	
PZA02564_2	C	33	0.28			100%
PZA02585_2	T	59	0.5	0.5	0.38	
PZA02585_2	A	59	0.5			98%
PZA02589_1	G	43	0.36	0.46	0.35	
PZA02589_1	A	77	0.64			100%
PZA02606_1	G	25	0.21	0.33	0.28	
PZA02606_1	C	95	0.79			100%
PZA02676_2	C	55	0.46	0.5	0.37	
PZA02676_2	T	65	0.54			100%
PZA02683_1	G	25	0.21	0.33	0.28	
PZA02683_1	T	95	0.79			100%
PZA02763_1	C	42	0.35	0.46	0.35	
PZA02763_1	T	78	0.65			100%
PZA02890_4	G	51	0.43	0.49	0.37	
PZA02890_4	T	69	0.58			100%
PZA02916_5	G	73	0.61	0.48	0.36	
PZA02916_5	A	47	0.39			100%
PZA02957_5	G	78	0.65	0.46	0.35	
PZA02957_5	T	42	0.35			100%
PZA03116_2	G	109	0.91	0.17	0.15	
PZA03116_2	T	11	0.09			100%
PZA03182_5	C	73	0.61	0.48	0.36	
PZA03182_5	T	47	0.39			100%
PZA03231_1	A	91	0.76	0.37	0.3	
PZA03231_1	G	29	0.24			100%
PZA03391_2	C	72	0.6	0.48	0.36	
PZA03391_2	A	48	0.4			100%
PZA03395_3	C	96	0.8	0.32	0.27	
PZA03395_3	T	24	0.2			100%
PZA03404_1	A	37	0.31	0.43	0.34	
PZA03404_1	G	83	0.69			100%
PZA03445_1	A	41	0.36	0.46	0.35	
PZA03445_1	T	73	0.64			95%
PZA03470_1	G	109	0.92	0.14	0.13	
PZA03470_1	T	9	0.08			98%
PZA03474_1	G	28	0.23	0.36	0.29	
PZA03474_1	C	92	0.77			100%
PZA03507_1	G	70	0.6	0.48	0.36	
PZA03507_1	T	46	0.4			97%
PZA03602_1	T	73	0.61	0.48	0.36	
PZA03602_1	A	47	0.39			100%
PZA03644_1	A	64	0.54	0.5	0.37	
PZA03644_1	G	54	0.46			98%
PZA03661_3	A	78	0.65	0.46	0.35	
PZA03661_3	G	42	0.35			100%
PZA03695_1	C	104	0.87	0.23	0.2	
PZA03695_1	T	16	0.13			100%
PZA03728_1	T	115	0.96	0.08	0.08	
PZA03728_1	C	5	0.04			100%
PZA03733_1	C	80	0.68	0.44	0.34	
PZA03733_1	T	38	0.32			98%
PZA03743_1	C	35	0.29	0.41	0.33	
PZA03743_1	G	85	0.71			100%
PZB00008_1	G	69	0.58	0.49	0.37	
PZB00008_1	A	51	0.43			100%
PZB00068_1	A	77	0.64	0.46	0.35	
PZB00068_1	G	43	0.36			100%
PZB00085_1	C	39	0.33	0.44	0.34	
PZB00085_1	T	79	0.67			98%

Marker name	Allele	Allele count	Frequency	Diversity	PIC	Call rate
PZB00109_2	G	38	0.32	0.43	0.34	
PZB00109_2	A	82	0.68			100%
PZB00175_6	G	14	0.14	0.24	0.21	
PZB00175_6	A	88	0.86			85%
PZB00232_1	G	80	0.67	0.44	0.35	
PZB00232_1	A	40	0.33			100%
PZB00772_1	T	69	0.58	0.49	0.37	
PZB00772_1	C	51	0.43			100%
PZB00869_4	T	68	0.58	0.49	0.37	
PZB00869_4	C	50	0.42			98%
PZB01042_7	T	24	0.2	0.32	0.27	
PZB01042_7	C	96	0.8			100%
PZB01156_2	C	37	0.34	0.45	0.35	
PZB01156_2	T	73	0.66			92%
PZB01186_1	C	52	0.43	0.49	0.37	
PZB01186_1	T	68	0.57			100%
PZB01358_2	G	92	0.77	0.36	0.29	
PZB01358_2	C	28	0.23			100%
PZB01400_1	T	105	0.88	0.22	0.19	
PZB01400_1	C	15	0.13			100%
PZB01647_1	C	38	0.32	0.43	0.34	
PZB01647_1	T	82	0.68			100%
PZB02017_1	T	93	0.78	0.35	0.29	
PZB02017_1	A	27	0.23			100%
PZB02033_2	C	6		0	0	
PZB02033_2		0	0			5%
PZB02155_1	C	43	0.36	0.46	0.35	
PZB02155_1	A	77	0.64			100%
PZB02283_1	G	83	0.7	0.42	0.33	
PZB02283_1	A	35	0.3			98%
PZB02480_1						0%
PZB02480_1						
PZB02510_5	A	76	0.63	0.46	0.36	
PZB02510_5	G	44	0.37			100%
PZD00022_6	A	46	0.38	0.47	0.36	
PZD00022_6	G	74	0.62			100%
PZD00027_2	C	31	0.27	0.39	0.31	
PZD00027_2	T	85	0.73			97%
PZD00054_1	C	62	0.53	0.5	0.37	
PZD00054_1	T	54	0.47			97%
PZD00072_2	T	83	0.69	0.43	0.34	
PZD00072_2	C	37	0.31			100%
sh1_2	A	98	0.82	0.3	0.25	
sh1_2	T	22	0.18			100%
umc128_2	G	44	0.38	0.47	0.36	
umc128_2	A	72	0.62			97%
ZHD1_1	C	37	0.31			
ZHD1_1	T	83	0.69			100%
AVERAGE				0.43	0.34	

