Phenotypic and Genotypic Characterisation of F_4 Families Derived From a Temperate X Tropical Maize Population

By

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ABSTRACT

Maize is a tropical crop that is adapted to diverse environments. However its production is compromised leading to poor grain yield, especially in sub-Saharan Africa (SSA) due to environmental challenges. Smallholder farmers in the region do not have capacity and resources to condition the production environments. This calls for need for breeding to improve maize adaptation to changing climate and challenging environments. Early maturing maize varieties should be richly endowed with early flowering and low grain moisture content. These plants are capable to escape drought that is lately devastating because of climate change. Therefore early maturing lines and hybrids need to be developed now and again to sustain maize production. The main objective of this study was to conduct phenotypic and genotypic characterisation of F_4 families that were derived from a temperate x tropical maize population in the maize programme at the University of KwaZulu-Natal, South Africa and the specific objectives were to (i) phenotypically characterize F_4 families derived from temperate x tropical maize F_2 cross, (ii) genotype F_4 families derived from temperate x tropical maize F_2 cross, (iii) select F_4 families for earliness using phenotypic data and genotypic data (background selection) and (iv) determine the relationship between grain yield and secondary traits in maize inbred families derived from temperate x tropical F_2 population. To phenotypically characterize the F4 families, the trials were laid out as 5 x 10 alpha lattice designs with two replications, at Cedara Research Station and Ukulinga Research Farm in KwaZulu-Natal, South Africa, during the 2015/16 summer season. Data was analysed using GenStat 17th edition and SAS version 9.3 computer package. There were significant differences at 0.1%. 1% and as well as 5% among families for all phenotypic traits indicating the presence of genetic variability. The families 15MAK8-74, 15MAK8-75 and 15MAK8-57 were among the top ten high yielding families on both sites but only 15MAK8-74 was early. It had lower anthesis date (AD) and silking date (SD) as well as the moisture content lower than 15.5%. Ear prolificacy and ear height were highly significant and strongly, positively correlated with grain yield in both sites. Therefore the genes controlling the two traits can be utilized for improvement of the test families in future breeding programmes. Ear height was the major contributor to grain yield through direct effects thus qualifying it to be selected to boost yield for target environments. To genotype the F_4 families, they were planted in a tunnel for seven weeks, replicated four times and 391 SNPs markers were used to genotype. Data was analyzed for genetic diversity using GenAlex software version 6.5. Cluster analysis was done using (UPGMA) in DARwin 5.0 software. The results indicated the effectiveness of the SNP markers to discriminate the families into three different clusters. The early maturing families were spread across the three clusters. Cluster III had four families allocated in it and three of these families were early maturing. Both molecular data and phenotypic data were

effective in revealing the variation among families in the temperate x tropical population, which can be exploited in breeding productivity maize inbred lines. In general, the study identified invaluable maize inbred families. Therefore this information may be utilized for future improvement and genetic conservation efforts of maize.

Keywords: correlation, early maturity, genetic diversity, maize, phenotypic selection.

DECLARATION

I, Sanelisiwe Mzobe, declare that

1. The research reported in this dissertation, except where otherwise indicated, is my original research.

2. This dissertation has not been submitted for any degree or examination at any other university.

3. This dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

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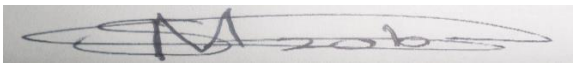

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Sanelisiwe Mzobe

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

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Prof. John Derera (Supervisor)

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Dr Julia Sibiya (Co-Supervisor)

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DEDICATION

I would like to dedicate this thesis to my loving and supportive mother, Mavis Mzobe and to my daughter, Liyakhanya Mbuyisa with lots of love.

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LIST OF ABBREVIATION

AD = anthesis date

AFLP = Amplified fragment length polymorphism

ASI = anthesis-silking intervals

cm = centimetre

CV = Coefficient of variation

DNA = Deoxyribonucleic acid

EH = ear height

EPO = ear position

EPP = number of ears per plant/ ear prolificacy

ER = ear rot

F1 = first filial generation

F_{is} = inbred coefficient

FRET = fluorescence resonant energy transfer

GA = genetic advance

GCV = genotypic coefficient of variation

GDD = growing degree-day

GM = grain moisture

GY = grain yield

H^2 = broad sense heritability

H_o = observed genetic diversity within genotypes

H_e = average gene diversity within genotypes

Indels = insertion and deletions

KASP = Kompetitive Allele Specific Polymerase Chain Reaction

MAS = Marker-assisted selection

N_a = total number of alleles per locus

N_e number of effective alleles per locus

NTCs = No template controls

PIC = Polymorphic Information Content

PCR = Polymerase Chain Reaction

PCV = phenotypic coefficient of variation

PH = plant height

RAPD = Random amplification of polymorphic DNA

RFLP = Restriction fragment length polymorphism

RL = root lodging

SD = silking date

SL = stem lodging

SNP = Single nucleotide polymorphism

SSA = Sub-Saharan Africa

SSR = Microsatellites/ Simple Sequence Repeats

t/ha = tonnes per hectare

UK = United Kingdom

UKZN = University of KwaZulu-Natal

YS = yield score

CHAPTER ONE

INTRODUCTION

1.1 Introduction

This chapter introduces maize as an important crop. It highlights the significance of maize in the world with particular focus on sub-Saharan Africa and outlines the constraints affecting maize production. Crop improvement through breeding based on the objectives mentioned below is presented as a method to circumvent the challenges to maize production and lastly the structure of the thesis is provided.

1.2 Significance of maize

Maize (*Zea mays* L.) is one of the most important crops worldwide, including South Africa. It is a tropical crop but has wide adaptation due to wide spread production and introduction into environments which were previously not suitable due to growing demands for food. It is now adapted to the broadest range of climate conditions from 58^{o} North (Canada) to 40^{o} South (Chile) (Bouchet et al., 2013), from the sea level in West Africa, through middle altitudes in southern Africa to the highlands in East Africa. The ability to grow in a wide range of environments is reflected in the high diversity of morphological and physiological traits (Paliwal, 2000b). In Africa, maize was introduced in the 1500s and since then it has stayed a dominant food crop for Africans (McCann, 2001). It is rated among the most important economic crops because of its high nutrition and is an important source of carbohydrate, protein, iron, vitamin B and minerals. In most parts of sub-Saharan Africa (SSA), maize is consumed as cereal and also used as a livestock feed, dry forage, silage or grain (Mienie and Fourie, 2013; Sibiya et al., 2013). Therefore it has an impact on global food security, feeding more than 1.2 billion people in SSA and Latin America. In developed countries, it is used as an animal feed and is also used for industrial application.

The crop is strategic in that all plant parts of maize have economical value to the industry and in particular to the households in SSA. In industry, it has wide range of uses; starting from food processing to non-food products (Keyser, 2006). At the household level in SSA, it contributes to about 30% of calories that are consumed by humans in southern Africa

(Magorokosho, 2007). In industrial food, it is used as an ingredient in food and drinks, such as corn syrup in soft drinks and maize meal. It produces a lot of starch which is used as food ingredient also in many foods. The same starch from maize produces alcohol including fuel ethanol after it had been fermented but that has been discouraged in SSA due to inadequate production of maize for food. With maize being used for a lot of things, Magorokosho et al. (2008) foresees that maize demand continues to increase in the world. However there is not adequate production of maize in Africa, south of the Sahara - that has limited its industrial use. This is because there is still a large gap between human consumption of maize and its production.

1.3 Challenges facing maize production

Maize production is lowest in Africa, south and east of the Sahara compared to other regions. The difference in production and yield of maize between 13 selected African countries (Angola, Congo, Ethiopia, Ghana, Kenya, Malawi, Mozambique, Nigeria, Somalia, Uganda, United Republic of Tanzania, Zambia and Zimbabwe) and other regions are shown in Figure 1.1 and Figure 1.2. The big gap between Africa and the United States in their production and yield is because in the United States they use single cross maize hybrids, which are high yielding whereas in Africa, farmers still use open pollinated varieties or three way crosses. These are associated with low yields. This has resulted in pressure on governments' agricultural sector transformation policies (Odendo et al., 2001).The other major constraints that contribute to maize low yield include increased pests and diseases pressure, unreliable annual rainfall amounts and also a lack of quality improved germplasm (Mhembere, 2007), which are compounded by poor management practices. Also market failure has an impact on low productivity which negatively feeds into the productive systems as a disincentive for farmers to produce more.

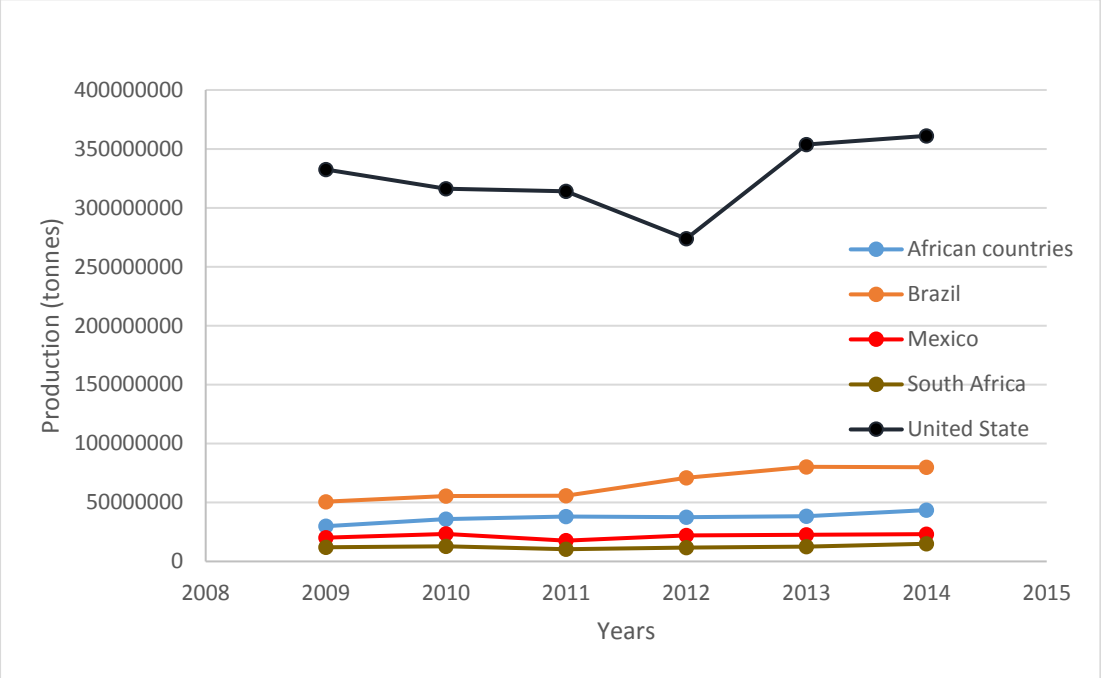

Figure 1.1: Global maize production (data source- FAOSTAT, 2016).

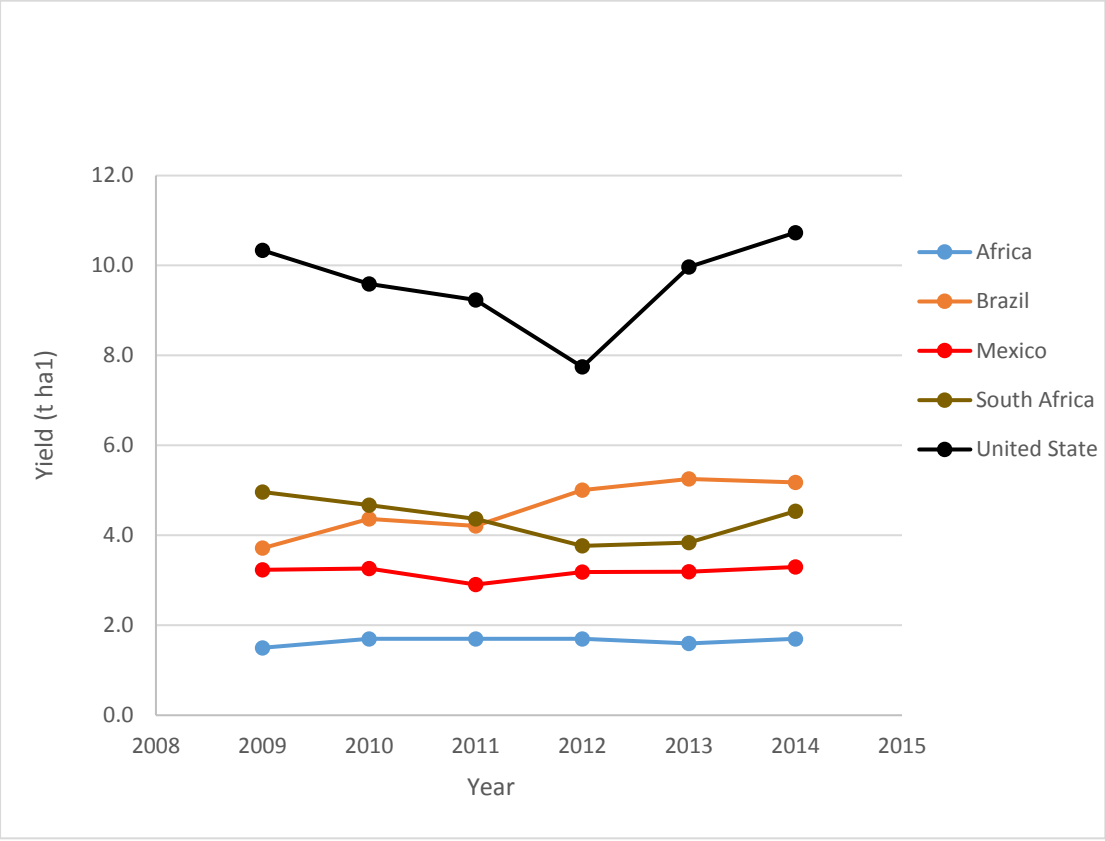

Figure 1.2: Global grain yield (t ha^{-1}) of maize (data source - FAOSTAT,2016).

1.4 Strategies for improving maize production in Africa

Several strategies can be considered to improve productivity of maize in Africa. Efforts such as biological and agronomic research and farmer support or extension services have been designed and implemented with the aim to improve maize productivity (Oyekale and Idjesa, 2009). Despite many efforts to increase production, maize still falls way short of demand (Sprague, 1982;Oyekale and Idjesa, 2009) partly due to low adoption of new technologies and limited access of them. Therefore, there is still need to develop new hybrids and make them easily accessible to the farmers (Raphael, 2014). However there are also other proposed solutions to the low productivity of maize in the sub-Saharan region. These are discussed in this section.

1.4.1 Access to key inputs

There is a need for farmers to use the right amount and quality of agricultural inputs in order to realise benefits of improved varieties in sub-Saharan Africa. Raphael (2014) suggested that high yielding hybrids have to be made available to farmers on time together with inputs like fertilizer since improved maize varieties need more fertilizer and pesticides for their growth. Drought is the major factor affecting the production and food security in SSA (Clover, 2003), therefore early maturing hybrids need to be given to farmers to help escape devastating drought. Soil fertility need to be addressed as it is the foundation of plant health. Crop response to numerous inputs will be restricted whenever any nutrient become limiting. These essential inputs need to be available at an affordable rate (Sprague, 1982).

1.4.2 Assurance of produce market

Another aspect that requires attention is the creation of demand for farmers maize produce. Farmers are only interested in feeding their families and producing enough maize for their own needs but with profits involved they can grow excess maize and sell (Sprague, 1982). With that assurance they will adopt superior quality seeds, inputs and practices to increase production (Kaliba et al., 2000). Therefore, the market must be in place to accommodate increased flow of product. The transport infrastructure have to be available to handle massively increased demands for services. All these need to be in place if farmers are to move with more serious production practices (Sprague, 1982).

1.4.3 Breeding early maturing maize

A genetic solution is required to improve maize productivity in SSA. In this regard, breeding and using early maize varieties with short growing seasons are an advantage. These cultivars are bred to stand the effects of reduced moisture supply during flowering and grain-filling period (Gasura et al., 2010). Farmers with these cultivars will not be at risk of losing all their maize, resulting in improved food security. Bello et al. (2012) reported that early maturing maize varieties are ideal for intercropping since they compete less for moisture, light and nutrients than late maturing ones. They offer flexibility in planting dates and enable multiple plantings in a season. They also avoid known terminal drought periods during cropping seasons. With these kind of varieties, maize production will be higher than what it is at present.

1.5 Rationale for the Research

Among other challenges climate change effects would impact negatively on maize productivity in the SSA, especially when less appropriate varieties are grown. Lately, climate change present challenges in food security in sub-Saharan Africa, revealed by global warming and increased incidence of drought and shortening of the growing season in maize production areas. This was evident during the 2014/15 and 2015/16 season in eastern and southern Africa, to which South Africa belongs. The increasing threat of climatological extremes including very high temperatures might lead to catastrophic loss of crop productivity and result in wide spread famine (Bita and Gerats, 2013). Therefore there is a need to develop maize varieties that can cope with climate change and other environmental challenges.

Development of early maturing varieties which can escape drought would be crucial to meet the food demands and ensure food security at household and national levels in SSA. In addition early maturing varieties fit in well in intercropping and rotation practices, and would mature within the short growing seasons. However, development of early varieties requires identification and generation of inbred lines carrying the earliness genes while they also have high yield potential. This study seeks to determine genetic diversity in the breeding germplasm population that can be used for developing early maturing maize varieties.

To achieve the objective, new maize inbred families were derived using conventional breeding methods from the temperate and tropical F_2 population. The temperate germplasm materials were introduced and incorporated into the tropical based germplasm with the intention of introducing new alleles for early maturity to fit the germplasm into the increasing short seasons in SSA and the resultant products would be ideal for late season planting and fit in the increasingly shortening agricultural seasons across the region.

1.6 Research objectives

1.6.1 Main Objective

The main objective of this study was to conduct phenotypic and genotypic characterisation of F_4 families that were derived from a temperate x tropical maize population in the maize programme at the University of KwaZulu-Natal, South Africa.

1.6.2 Specific Objectives

The following specific objectives were pursued in the study:

a) To phenotypically characterize F_4 families derived from temperate x tropical maize F_2 cross.
b) To genotype F_4 families derived from temperate x tropical maize F_2 cross, using 391 SNP markers.
c) To select F_4 families for earliness using phenotypic data using genotypic data (background selection).
d) To determine the relationship between grain yield and secondary traits in the maize inbred families derived from a temperate x tropical F_2 population.

1.7 Outline of the dissertation

The dissertation is made up of six chapters that are outlined in this section.

Chapter 1: Introduction

This section covers the importance of maize; information on maize constraints; the rationale of the research, objectives and the outline of the dissertation.

Chapter 2: Literature review

The body of knowledge for the research context is presented and discusses the importance of maize. It also reviews literature that is pertinent to maize production, constraints of maize production, early maturity, maize genetic diversity, classical breeding as well as marker-assisted selection, phenotypic selection for grain yield and early flowering, and the relationship between grain yield potential and secondary traits. Conclusions drawn from the review are provided.

Chapter 3: Materials and Methods

The materials and methods chapter describes the germplasm and other material resources, preparation and methods that were used to execute the study.

Chapter 4: Results

Present the results showing all analysed parameters and their relation in tables and figures.

Chapter 5: Discussion

This section provides interpretations for the results and further explains the data presented under the results section.

Chapter 6: General overview of the study findings

The section provides an overview of research findings, conclusions and implications for breeding, and recommendations for future study.

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CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter reviews the present body of knowledge for the research context and discusses the importance of maize. It also reviews maize production, constraints of maize production, maize genetic diversity, classical breeding as well as marker-assisted selection, phenotypic selection for yield and flowering dates, early maturity and the relationship between grain yield and secondary traits. Conclusions drawn from this review are provided at the end of the chapter.

2.2 Global importance of maize

Maize (*Zea mays* L.) is truly a remarkable crop species and is adapted to wide range of environmental conditions. It feeds 1.2 billion people in Africa and Latin America, while it is a staple crop in all the sub-Saharan African (SSA) countries (Mienie and Fourie, 2013; Sibiya et al., 2013). Its grains are rich in vitamins A, C and D, carbohydrates and essential minerals and contain 9% protein (Shiferaw et al., 2011) which are important for immune system and growth. It contributes about 30% calories consumed in Southern Africa (Magorokosho, 2007). Maize grains are also rich in dietary fibre and calories which are a source of energy.

Despite it being a food crop, it also has wide range of industrial applications. The industry has strong linkages throughout the economy, both upstream to the input industries and downstream into milling, animal feed and food processing industries. For industrial food, it is used as an ingredient in food and drinks like maize syrup in soft drinks and maize meal (White, 1994). Maize contains starch which is used as food ingredient as well. The same starch from maize produces alcohol including fuel ethanol after it had been fermented. Paper industry also uses maize so much (Hallauer and Carena, 2009).

2.3 Maize production

With maize demands growing year after year because of it numerous uses, it is now produced in many different regions and countries. It is currently produced at nearly 100 million hectares in 125 developing countries (CIMMYT, 2016). The demands for maize is likely to double by 2050 (Van Wart et al., 2013) but production may fail to meet demand due to limiting factors such as biotic and abiotic stresses (Shiferaw et al., 2011). African maize yields are much lower compared to the rest of the world (FAOSTAT, 2016). Currently, grain yield is averaging as low as close to 1.6 t h^{-1} in Africa posing a serious threat to food security whereas in the United State it is 9.6 t h^{-1} (FAOSTAT, 2016). The high yields obtained in the United State of America show that maize has high potential under high input farming systems that is used in the United States. High temperature and erratic weather conditions are the most common factors reducing maize productivity in Africa followed by lack of improved seed. In spite of the factors that threatens maize production, the favourable alleles in the global maize germplasm that contribute to higher yield will always be there but the issue is that these alleles will be scattered over a wide array of land races and populations (Prasanna, 2012).

In South Africa, maize is grown throughout the country commercially on large farms and on small farms. It has an average production of approximately 10 to 12 million tonnes per annum in the past years. In 2016 it stands at approximately 7.7 million tonnes, down by 27% from the previous years (FAOSTAT, 2016). The steep decline is mainly attributed to the EL Nino-related drought conditions. With drought causing much loss in the production, South Africa is mounting with food prices, with the cost of maize rising by about 75% over the past year (2015). Improved input needs to be used more and effectively to escape this much loss in the production of maize.

2.4 Constraints of maize production

With the productivity of maize declining more and more in Africa, it is not only due to poor farming system but there are more other factors. The major constraints that contribute to maize low yield and causing the gap between its demands and its production, are attributable to increased pests and diseases, unreliable annual rainfall amounts and also a lack of quality improved germplasms (Mhembere, 2007).

2.4.1 Biotic Constraints

Maize is subject to attack by a number of biotic factors such as occurrence of pests like stalk borers and diseases such as stalk rots. Stalk borers are present wherever maize is grown, the caterpillars feeds on the stock or maize ear and that results in low yields, on the other hand stalk rots cause wilting of the plants before or after pollination thus decreasing the yield. These vary from one environment to another. The problem of pests and diseases attack on crops is a major challenge facing farmers as their incidence often leads to the reduction in the quality of the crop produced and it usually results in huge loses which discourage farmers from continuous production of the crop.

Maize endemic diseases are difficult to control as their occurrence cannot be predicted. They change year after year depending on the weather conditions (Relman et al., 2008). The impact of most diseases is also related to geographical or climatic factors which favour the growth of a plant (Hughes, 2006). These diseases occur at any stage of maize growth as long as the conditions are favourable for them, thus can also be found during the favourable conditions of maize growth and crucial stages like flowering stage and they could cause a huge damage (Sibiya, 2010).

2.4.2 Abiotic Constraints

Other devastating factors that contribute to low yields are the abiotic factors, which are more of the environmental conditions and occur naturally. Principal abiotic factors that affect maize are water and nutrient limitations. Water shortage may lead to drought which affects maize by slowing growth leading to stunted plants with low yields. In most cases, small-scale farmers depend on the rainfall in their production areas. When their areas are subjected to drought or very low rainfall and there is no irrigation system to back it up, the yield becomes low (Srinivasan et al., 2004; Sibiya, 2010). Drought is more devastating during flowering stage. Flowering stage is when the yield potential is set and that is when the yield drops to its lowest when the plant experiences any kind of stress (Srinivasan et al., 2004), that the yield is reduced by 6-8% for each day of stress (Colless, 1992).

Another principal abiotic factors which is nutrients limitation begins when a source-sink balance is moved. Source-sink balance is the mechanism by which nutrients are conveyed through the phloem, from sources to sinks. At the sources (leaves), the photo-assimilates

are moved into the sieve elements (phloem cells) through active transport. Maize has high nutrients demands especially nitrogen (N), phosphorus (P) and potassium (K) (Birch et al., 2003), if these are no longer moving in a source-sink manner, a plant starts experiencing stress and a stressed plant does not reproduce the way it should and that leads to yield loss.

Even more worrisome is that temperatures are no longer stable, they are changing all the time. Temperature plays a very important role in the growth of maize plants, if the temperature requirements are not reached the plants get affected negatively. Factors such as photosynthesis, translocation and pollen viability (Lafitte, 2000), kernel growth, kernel mass and protein accumulation (Jones et al., 1985; Monjardino et al., 2006) get affected and then the yield drops. When temperatures are high during pollination, the pollen viability is decreased (Dupuis and Dumas, 1990), while low temperatures cause freeze damage to a plant especially when it is still at seedling stage.

These environmental issues are even hard to control because they occur naturally. Soil acidity is another factor that decreases maize yields lately with the occurrence of acid rains. Maize is very sensitive to soil acidity with the optimal soil pH being between 5.5 and 6.5. However, most of soils under smallholder farming are acidic or highly basic. That results from improper use of fertilizer, erosion or when the soils are generall under saline or acidic conditions. The productivity of maize decline with increasing salinity (Lafitte, 2000; Yensen and Biel, 2008). When maize is exposed to salinity at seedling stage, the vegetative growth is reduced, tasselling and silking get delayed and the cobs become small with fewer kernels (Kaddah and Ghowail, 1964). This then results in yield decreases.

The use of unimproved varieties is the other factor that lead to production failure and low yield. These varieties fail to adapt to the current conditions. However the current study focuses on development of early maturing maize which can be deployed in areas that are prone to abiotic stress.

2.5 Early maturing maize

Development of early maturing maize will minimise the gap that is between the production and consumption of maize. The term early maturing maize refers to the maize varieties that

take a short period of time to reach 50% anthesis and physiological maturity. The early maturing maize is classified by it shorter plant height, less number of leaves, and lower grain yield. The yield is low when the plant population is low but when plant population is increased, the grain yield also increases (Hunter, 1977).

The use of early maturing maize will sustain food security in SSA where maize is the staple food crop and where drought is persisting. Varieties that mature early provide early harvests and are ideal for food security. These varieties do not just provide food early in the season but they are also capable of escaping late season drought (Gasura et al., 2014). Early maturing maize variety plays a great role in the food and farming systems of smallholder farmers, offering flexibility in planting dates. It is also ideal for off-season plantings in drying riverbeds. It enables multiple planting in a season, avoiding the risk of losing a single crop due to drought.

Improved early maturing maize varieties will give an assurance for increased maize productivity and will result significantly to the expansion of maize into new edges which have the greatest potential for increased maize production. Early maturing maize is also ideal for intercropping as it provides less competition for moisture, light and nutrients (Pswarayi and Vivek, 2008). Earliness is a very important trait for many tropical maize cultivars for a brief rainy season to sustain the yield.

2.6 Genetic diversity in maize

The information on genetic diversity is very crucial for germplasm development. Genetic diversity is the variability among plants due differences in the genetic make-up that encode specific traits and it can be transmitted from one generation to the next (Acquaah, 2007). The environment has an influence on the degree of expression of genes. Genetic diversity is created through hybridization and recombination, mutation and modification of chromosome number and structure (Hamrick et al., 1992; Acquaah, 2007).

Assessing genetic diversity in crops plays an important role in crop improvement programmes and conservation of genetic resources through the identification of variation of individuals or populations (Hallauer and Miranda, 1988; Jarvis et al., 2005; Semagn et al.,

2012). The genetic diversity among and within landraces makes them valuable resources as potential donors of genes for the development and maintenance of modern crop varieties.

Studying phenotypic and genetic diversity is very important in maize breeding. It assists with (i) identifying groups with similar genetic background; (ii) maintaining and broadening the genetic base of the elite lines; (iii) introgression of genes into a population and development of new hybrids and; (iv) generating segregating progenies with maximum genetic variability for later selection (Jarvis et al., 2005; Magorokosho et al., 2006).

Crop domestication and artificial selection resulted in the loss of genetic diversity in cultivated crop species when compared to their wild species. The loss was not equally experienced by the genes in the genome during domestication. Those genes that do not control favorable traits, the loss is basically a function of the size of the bottleneck of the environment. However, the loss is severe for genes of desired traits (Yamasaki et al., 2007). In addition, Tanksley and McCouch (1997) affirmed that most of the diversity in maize is poorly understood and underutilized in modern crop improvement programmes due to the difficulty of identifying useful genetic variants buried in the background of low yielding varieties and landraces. Therefore genetic diversity studies still need to continue to sieve through the variation in the maize gene pool and build up the understanding of how they impact phenotypes of agronomic importance especially for marginal production environments. Such knowledge will be useful for producing high productive inbred lines which in turn will be used to develop superior hybrids (Cholastova et al., 2011).

2.7 Estimation of genetic diversity

For genetic diversity analysis, there are different kinds of data that have been used. These include pedigrees, eco-geographic data, morphological, biochemical and molecular data (Hinze et al., 2005). A widely used measure is the co-ancestry coefficient (f), calculated from pedigree records, defined as the probability that two homologous genes drawn at random from two individuals are identical by descent (Reif et al., 2005). In maize breeding, the pedigree information is used to allocate newly developed inbred lines to heterotic pools (Messmer et al., 1993).

Alternatively, the genetic similarity or distance between genotypes can be assessed with DNA markers. Markers have been employed to study genetic diversity and have shown existing variation among individuals and populations (Jarvis et al., 2005). The simple sequence repeats (SSR) markers are mostly used to characterize crop germplasm due to them having high degree of variability and therefore they are suited for population studies. They are capable of distinguishing and identifying closely related genotypes (Ibitoye and Akin-Idowu, 2011). When analysing genetic diversity it is important to pay attention to (i) sampling strategies; (ii) utilization of various data sets; (iii) choice of genetic distance measure; and (iv) objective determination of genetic relationships (Franco et al., 2001).

2.8 Classical breeding

The central basis of plant breeding is to select specific plant traits considered important by plant breeders. Classical breeding entails selection of plants conveying desired traits to be used as parents in a crossing design to generate hybrids (Nadarajan and Gupta, 2010). This method of breeding relies largely on homologous recombination between chromosomes to create genetic diversity (Johnson, 2000) then selecting from a plant's natural complement of genetic elements (Ulukan, 2011). All in all, the goal is to change many traits simultaneously, (Johnson, 2000). Selected offspring are grown and tested in following years (Rao and Hodgkin, 2002). This process takes long and it requires a lot of money. This is so because classical breeding is somehow very complex with large size of populations that result from the crosses.

With new technologies such as MAS, the complexity of classical breeding have been simplified. Markers work best when breeding for polygenic or quantitative genetic traits that have been developed. In that way complexity can be simplified and therefore classical breeding becomes much broader in scope and potential than it was in the past. Classical breeding still stands as the most effective approach especially when dealing with traits controlled by multiple genes (Xu and Crouch, 2008).

2.9 Marker-assisted selection

Marker-assisted selection (MAS) is a method that is mostly used to assist classical breeding. They indirectly selects desirable individuals in a breeding scheme based on DNA molecular

marker patterns rather than on their observable traits (Rosyara, 2006). Collard and Mackill (2008) and Ibitoye and Akin-Idowu (2011) stated that markers determine the genetic make-up of a plant and also represent or compare genetic differences between individual organisms. They are strongly allied with agronomical important genes, they assist in the selection of elite lines for the next generation crosses or progenies with the presence of the gene on interest (Xu et al., 2004).

Moreover, MAS method improves the precision and speed of conventional breeding strategies (Collard and Mackill, 2008). The use of this method helps breeders better understand the genetic and genomic control of the agronomic traits and thereafter design more efficient breeding strategies (Steele et al., 2004).

2.10 Markers that have been used to measure diversity

There are different markers that have been used to measure and interpret diversity in different organisms. These markers have been developed into many systems based on different polymorphism-detecting techniques or methods (Jiang, 2013). Among the used markers, there is Restriction fragment length polymorphism, Amplified fragment length polymorphism, Random amplification of polymorphic, Microsatellite polymorphism and Single nucleotide polymorphism. These markers have advantages over other kind of methods of determining genetic diversity. They show differences on a more detailed level without interferences from environmental factors. Molecular markers provide fast results, detailing genetic diversity.

2.10.1 Restriction fragment length polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) are markers that refers to a difference between two or more samples of homologous DNA molecules. These DNA molecules arise from differing locations of restriction sites (Xu, 2010). The restriction enzymes break down the DNA sample which then result in restriction fragments being separated according to their lengths by gel electrophoresis. The digestion of purified DNA with the use of restriction enzymes that cut the DNA strand where there is a recognition site sequence. This leads to the formation of RFLPs that yield a molecular fingerprint that may be unique to a particular individual.

RFLPs are powerful markers. They were originally developed for mapping human genes. They then proved their usefulness in almost all species (Botstein et al., 1980). The development of RFLP markers lead to accelerated construction of molecular linkage maps for many organisms. It also improved the accuracy of gene location and reduced the time needed to construct a complete linkage map (Xu, 2010). In maize RFLPs have been used extensively and successfully for polymorphism justification at DNA level among populations.

2.10.2 Amplified fragment length polymorphism (AFLP)

Amplified fragment length polymorphism (AFLP) markers are used to detect DNA polymorphism. They are based on the selective PCR amplification of restriction fragments from a total double-digest of genomic DNA. They have reliably been used for determining genetic diversity and phylogenetic relationship among closely related genotypes. AFLP method combines the power of RFLP with the flexibility of PCR-based markers (Xu, 2010; Abdel-Mawgood, 2012; Adawy, 2014). With this type of markers restriction enzymes are used to digest genomic DNA followed by ligation of adaptors to the sticky ends of the restriction fragments (Adawy, 2014). AFLP products can be separated in high resolution electrophoresis systems. AFLP markers are applicable to all species, giving very reproducible results (Abdel-Mawgood, 2012).

2.10.3 Random amplification of polymorphic DNA (RAPD)

Random amplification of polymorphic DNA (RAPD) were the first PCR based molecular markers technique developed and they are by far the simplest (Williams et al., 1990). RAPD products are visualized on agarose gel stained with ethidium bromide. RAPD markers are easily developed and due to it being based on PCR amplification followed by agarose gel electrophoresis, they are quickly and readily detected.

RAPD do not need any knowledge of the DNA sequence for targeted gene since the primers binds somewhere in the sequence but the position is not exactly known (Adawy, 2014; Derera, 2015). This makes the method popular for comparing the DNA of biological systems that have not had the attention of the scientific community. They have much lower resolving power than targeted species specific DNA comparison methods due to them being dominant. They give less information per locus studied. RAPD are now mostly used to

characterize and trace the phylogeny of diverse plant species (Derera, 2015), but back then were broadly used in studying genetic diversity between plant species (Abdel-Mawgood, 2012), Due to their reproducibility problem they have been replaced with microsatellite markers which are currently the system of choice for maize genetic diversity.

2.10.4 Microsatellite polymorphism

Microsatellites are also called Simple Sequence Repeats (SSR) markers. These type of a markers are tandemly arranged into blocks of short nucleotide sequences which are usually 1-10 nucleotides long, repeated up to 50 times (Reddy et al., 2002; Xu, 2010). One of the most important attributes of SSR loci is their high level of allelic variation, making them valuable as genetic markers. The unique sequences produced by SSRs provide templates for specific primers to amplify the SSR alleles via PCR. SSRs are highly reproducible, co-dominant and multiallelic with expected heterozygosity frequently greater than 0.7 which then allows precise discrimination even of closely related individuals. They are easily analysed and are highly polymorphic due to the high mutation rate affecting the number of repeat units (Xu, 2010;Adawy, 2014).

When estimating genetic similarity among maize inbred lines, SSR markers have shown the highest level of polymorphism per single marker locus (Drinic et al., 2002), due to their codominant nature and high number of alleles per locus. By that it can also be used to discriminate highly related germplasms.

2.10.5 Single nucleotide polymorphism (SNP)

Single nucleotide polymorphism (SNPs) are individual nucleotide base markers. They outline the difference between two or more DNA sequences. These differences occur within a genetic sequence (Xu, 2010). SNPs are allocated according to nucleotide substitution as either transitions (C/T or G/A) or transversions (C/G, A/T, C/A or T/G) (Xu, 2010; Derera, 2015). They are the most widespread type of sequence variation in genomes revealed so far (Collins et al., 1998).

SNPs are numerous, more stable, potentially easier to score when compared to SSRs. In plants, SNPs are swiftly replacing SSRs as the DNA marker of choice for applications in

plant breeding due to them being more profuse, stable, amenable to automation, efficient and increasingly cost-effective (Edwards and Batley, 2010; Bolger et al., 2014). SNPs may fall within coding sequences of genes, non-coding regions of genes or inherent frequencies in different chromosome regions. Genetic maps are used for SNPs to align sequence data to their respective chromosome positions (Derera, 2015).

In breeding programmes, SNPs markers are used for numerous application such as, (i) marker assisted and genomic selection; (ii) association and QTL mapping; (iii) positioning cloning; (iv) haplotype and pedigree analysis; (v) seed purity testing; (vi) variety identification; and (vii) monitoring the combination of alleles that perform well in target environments (Xu and Crouch, 2008; Jannink et al., 2010; McCouch et al., 2010). In the current study, SNP markers will be used to determine genetic diversity and distance among inbred families for selection.

2.11 Phenotypic selection

Phenotypic selection is a method that occurs when individuals with different characteristics differ in their survival or maturity success. The idea of phenotypic selection started back in the days and used by Darwin and Wallace and it is widely accepted as the primary cause of adaptive evolution within natural populations (Kingsolver and Pfennig, 2007).

So for selection to take place there must be variation in the population that is either natural or induced, whereby individuals differ in some of their characteristics and differential reproduction. Some individuals are more adapted compared to others due to their distinctive characteristics. When the characteristics being selected show inheritance, selection leads to evolutionary change in those characteristics (Kingsolver and Pfennig, 2007).

Phenotypically selected individuals are analysed so as to determine their relationship with grain yield. The analysis provides means of estimating the strength of direct and indirect selection on individual traits (Kingsolver et al., 2001). Selection between different habitats provides an information of the traits adaptation in a particular habitat and that may lead to population differentiation (Wade and Kalisz, 1990). The selection vary spatially and temporally for morphological traits (Kalisz, 1986;Kelly, 1992). During selection, other

characteristics may change, together with the improved trait. After selection for earliness, for example, plant and ear height decreases (Nyhus et al., 1989), and this could be due to gene frequency changes.

With earliness in maize, the selection is carried out by phenotypically selecting for individuals which flower early with low grain moisture content. The selection depends on the shape and temporal position of the distribution of flowering times of a species as this is modified by the pollinator (Bierzychudek, 1981). During a flowering season pollen limitation seems to be more important for fertility than resource limitation. Flowering is related with reproductive success (Forrest and Thomson, 2010). Therefore timing of reproduction has great impact on when a plant will flower. Flowering date is one of the several traits which had showed significant genetic variation. Flowering dates are also used to select genotypes which are high yielding since they are easily measurable and could be used directly to increase yield.

Furthermore grain moisture is another important trait for early maturing maize. Therefore to analyse it, maize yields are standardized to 15.5%, then grain moisture is obtained by removing full length of several rows of maize kernels randomly. Selected ears from each row sampled and thoroughly mix the grain moisture, the moisture content is then established with an accurate moisture determination system (Lauer, 2002). Besides measuring with machine directly, husk senescence has often been proposed as an indirect selection criterion for grain moisture in maize inbred lines (Sweeney et al., 1994). These traits are analysed so as to detect their relationship with grain yield, so grain yield selection depends on secondary traits that have a strong relationship with it.

2.12 Importance of secondary traits

Secondary traits are those traits that are used as a selection criterion in crop breeding. Their use has shown to improve overall selection efficiency, probably by more than 20% especially under severe stress caused by drought and low Nitrogen (N) (Edmeades, 1996; Bänziger and Lafitte, 1997). Secondary traits have been successfully used to improve the level of genetic improvement in maize populations under abiotic stresses (Betrán et al., 2003). For a secondary trait to be useful in a programme, it must comply with several requirements (Lafitte et al., 2003; Royo et al., 2005): the secondary trait must be (i) genetically variable, with a genetic association with grain yield, (ii) moderate to high heritability under stress to

show that the trial is less affected by the environment, (iii) cheaper or faster to measure than grain yield, (iv) able to be observed at or before crossing time, (v) able to provide an estimate of yield potential before final harvest, and (vi) stable over time. It is important that the selective traits are not related with poor yield under non-stressed environment and are related to productivity rather than survival mechanisms (Lafitte et al., 2003).

2.13 The relationship between grain yield and secondary traits

Secondary traits offer an indirect way to select for yield because they have high heritability and are less complex. Due to their association with grain yield, their selection may indirectly improve grain yield with appropriate selection strategies (Srećkov et al., 2010; Badu-Apraku et al., 2014). It is known that the whole aim of breeding is to develop superior genotypes for grain yield and adaptation to different stress factors (Bello and Olaoye, 2009). However, grain yield is a complex trait and is difficult to directly select, thus secondary traits assist with the selection.

The traits are selected simultaneously; therefore having information on the genetic relationship between them is useful (Ramalho, 2000). This is so because improving one trait might change the expression of another trait due to being interrelated (Brichette et al., 2001; Wolf, 2003), so knowledge of association will clearly give an indication of which traits must be improved and which traits must be compromised. With that information it becomes easy to establish the selection criteria and the strategy to use to improve yield without compromising other important traits (Hallauer et al., 2010; Bernini and Paterniani, 2012).

Grain yield is decreasing each year due to drought, and will continue to decrease more as drought is becoming severe because of climate change. Therefore, breeding for drought tolerant varieties is the goal of many breeders. However, phenotyping for this kind of stress is difficult and needs accurate characterization of the traits involved in a tolerant variety (Ziyomo and Bernardo, 2013). Under drought, grain yield is strongly correlated with plant height, chlorophyll content and leaf senescence (Ziyomo and Bernardo, 2013). This is in contrast with Ribaut et al. (2009) who found that ears per plant (EPP), kernels per plant, kernels per ear, anthesis date (AD) and anthesis-silking interval (ASI) only have the strongest genetic correlation with grain yield. The leaf senescence, chlorophyll concentration and plant height are only moderately correlated with grain yield. They only agree with ASI

being a reliable indicator of drought tolerance as it has the strongest genetic connection with grain yield. The difference between their findings is mainly due to different germplasm used and the environment that is why there is a need to evaluate genotypes under different environments to determine the effect of genotype X environment interaction (GXE) on the correlation between yield and secondary traits.

For early maturing maize, grain yield is associated with certain traits as well. Grain-filling rate (GFR) and effective grain-filling duration (EGFD) are important physiological traits of grain yield formation. Therefore using them as secondary traits can help with the selection for high grain yield in early maturing maize (Gasura et al., 2014). Overally, understanding the level of expression of secondary traits in inbred lines and their hybrids and their correlation with GY, and identifying inbred lines with desirable alleles for direct or recycling purposes can add efficiency to the improvement of genotypes performance.

2.14 Conclusion

Agriculture is faced with different challenges which lead to decreased yield and food insecurity. Drought is the most devastating challenge among them all and its intensity varies significantly with climatic factors and other demographic factors. Researchers have proposed various characteristics related to drought resistance that could be used in selection and breeding programme. However, comprehensive understanding of the physiological and genetic basis of adaptation in moisture stress condition is still lacking. This calls for integration of different strategies to improve drought resistance germplasm, which is the development of more early maturing maize inbred lines and hybrids. Therefore early maturing varieties need to be given much attention since they provide hope to sustain food security under drought even though they has not been much yield that has been reported when using them, but they can close the gap between production and utilization of maize when grown in a large population.

The use of conventional breeding alone is not sufficient for effective plant breeding programmes that aim for highly adapted elite lines in a shorter space of time. The use of molecular marker technology can greatly assist by reducing generation times nearly by half. Markers are able to detect diversity at DNA sequence level, thereby informing the breeder of any desirable variation or genes. Therefore genetic diversity of genotypes needs to be

studied in depth for successful development of superior hybrids with the availability of powerful tools which are markers. Maize breeding relies on the available genetic diversity, thus better hybrids of maize need to be developed by making use of information on the genetic relationships and diversity among elite materials, which is of fundamental importance in hybrid crop improvement.

Phenotypic traits can also be used to distinguish between lines, even though they do not always reflect the genetic constitution in maize due to environmental effects. But using phenotypic method together with molecular marker method is the best strategy of breeding. Therefore there is a need to use these two methods together so as to determine their effectiveness. In the present study the molecular markers have been integrated with phenotypic method in determining earliness among inbred families.

The secondary traits highly associated with yield need to be identified especially those that are correlated with yield under stress conditions.

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CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

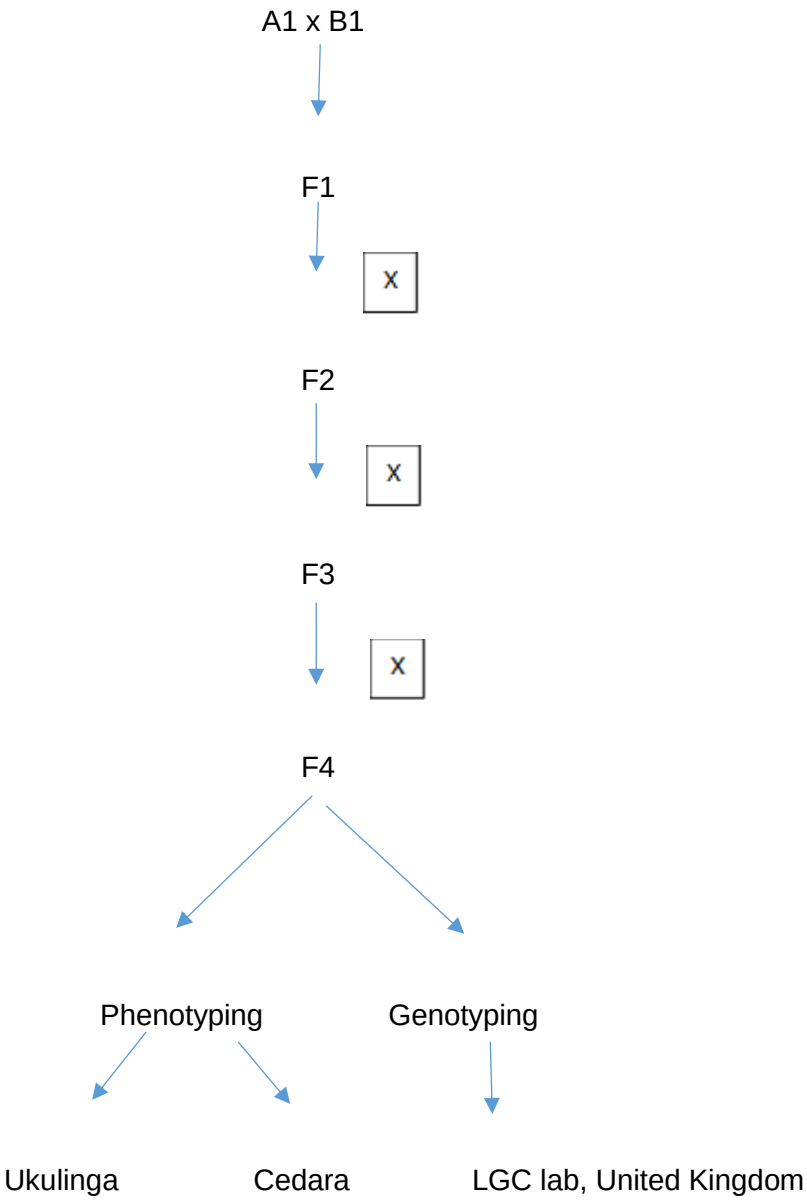
Temperate USA maize germplasm line with early physiological maturity genes were crossed with African adapted maize germplasm line that is high yielding but matures very late in the growing season. The crosses were done so as to incorporate the genes for earliness into this high yielding African population. The progeny F_1 cross has undergone selfing three times until it reached the F_4 generation that was used in the current study. The F_4 families were phenotyped and genotyped.

3.2 Germplasm

Fifty (50) new F_4 inbred families were derived from tropical x temperate population at the UKZN maize programme (Table 3.1). The F_1 plants were self-pollinated to advance to the F_2. The F_2 plants from the selfed F_1 plants were planted at the Ukulinga Research Farm for advancement to the F_3 during the 2015A (i.e. in summer; November 2014 – April 2015). These F_2 plants were self-pollinated to produce F_3 families. In 2015B (i.e. in winter: May – October 2015), the F_3 plants were planted at the Makhathini Research Station, in Jozini and were self-pollinated to produce F_4 families (Figure 3.1). The F_4 inbred families were then used for the current study.

Table 3.1: Inbred families used in the study.

Line	Name	Line	Name	Line	Name	Line	Name
1	15MAK8_1	14	15MAK8_35	27	15MAK8_85	40	15MAK8_130
2	15MAK8_4	15	15MAK8_44	28	15MAK8_88	41	15MAK8_135
3	15MAK8_5	16	15MAK8_45	29	15MAK8_89	42	15MAK8_141
4	15MAK8_7	17	15MAK8_46	30	15MAK8_90	43	15MAK9_5
5	15MAK8_11	18	15MAK8_57	31	15MAK8_91	44	15MAK9_6
6	15MAK8_14	19	15MAK8_58	32	15MAK8_93	45	15MAK9_7
7	15MAK8_17	20	15MAK8_59	33	15MAK8_97	46	15MAK9_10
8	15MAK8_19	21	15MAK8_74	34	15MAK8_99	47	15MAK9_12
9	15MAK8_23	22	15MAK8_75	35	15MAK8_103	48	15MAK9_14
10	15MAK8_30	23	15MAK8_76	36	15MAK8_104	49	15MAK9_16
11	15MAK8_31	24	15MAK8_78	37	15MAK8_105	50	15MAK9_27
12	15MAK8_32	25	15MAK8_81	38	15MAK8_125		
13	15MAK8_33	26	15MAK8_84	39	15MAK8_129		



Key: X represent self-pollination.

Figure 3.1: An illustration of how the maize inbred familes used in the study were produced.

3.3 Experimental sites

A total of 50 families were selected randomly and were evaluated across two sites in KwaZulu-Natal during 2015 to 2016 summer season. The sites used were Ukulinga Research Farm and Cedara Research Station. They are both located in Pietermaritzburg area but with different geographic conditions (Table 3.2). At Ukulinga, the planting was done on the 27th of November 2015 and at Cedara it was done on the 10th of December 2015.

Table 3.2: Geographical cordinates and environmental conditions of the study sites.

Location	**Latitude**	**Longitude**	**Altitude m.a.s.l**	**Total season rainfall (mm)**	**Temperature range (^{0}C)**
Ukulinga	29.67S	30.41E	809	447.30	9.32-41.44
Cedara	29.54S	30.26E	1068	508.28	8.81-39.47

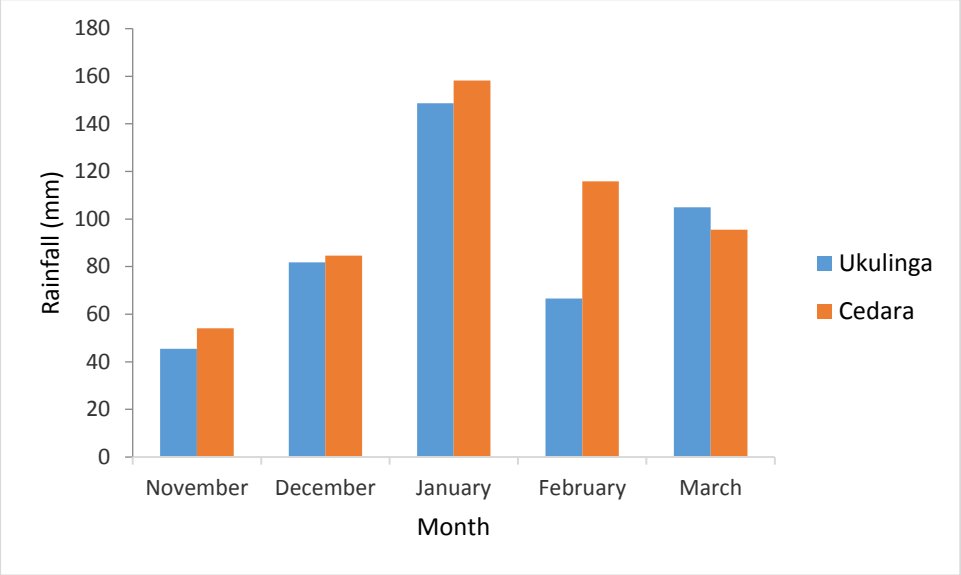


Figure 3.2: Total rainfall for Ukulinga and Cedara fo the duration of the study.

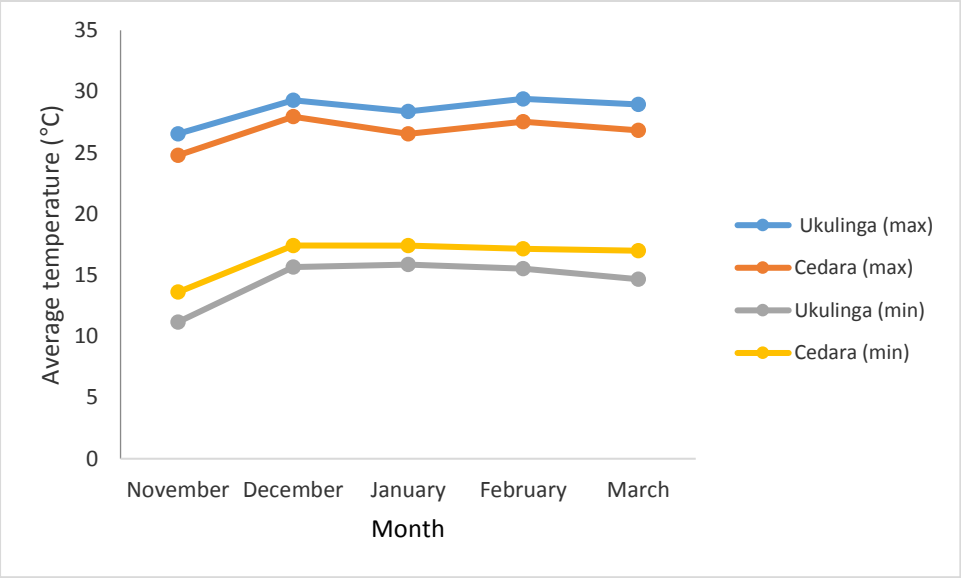


Figure 3.3: Average temperture of Ukulinga and Cedara for the duration of the study.

3.4 Phenotyping

3.4.1 Field trial design

In the two sites (Ukulinga and Cedara) that were used for evaluation, the plants were laid out in a 5 x 10 alpha lattice design with two replications. Each family was planted in one row with two seeds per hole. Each row was 5 m long. The inter-row plant spacing was 0.9 m and the in-row was 0.3 m to give a plant population of 100 plants. Two border rows were planted at the beginning and at the end of each block.

3.4.2 Field management

The experiments were conducted under rain fed conditions at both sites. The distribution of monthly rainfall for the growing season is presented in Figure 3.2 and temperature data is presented in Figure 3.3. Granular compound fertilizer (NPK) of 250 kg/ha was applied as basal before planting. These three elements are the main nutrients required in the development of healthy and productive plants. Nitrogen help plants grow quickly and better the quality of leaf. Phosphorus supports the formation of oils, sugars and starches. Potassium assists in photosynthesis, the building of protein and the reduction of diseases.

Immediately after planting the herbicides gramoxone (130 ml), dual (40 ml), callisto (10 ml) and karate (20 ml) were applied to control weeds. This was improved by hand weeding to keep the fields free of weeds throughout the season. After four weeks of planting, after crop emergence, plants were thinned to a single plant per hole and LAN top dressing fertilizer (28% N) was applied at a rate of 250 kg/ha to supply essential nutrients. The insecticide granules were applied on the maize leaves for stalk-borer control.

3.4.3 Data Collection

The following traits were measured following standard protocols used at CIMMYT (Magorokosho et al., 2008):

a. **Grain yield**: measured as grain mass per plot adjusted to 12.5% moisture content of grain harvested.

b. **Number of ears per plant**: measured by counting the number of ears per plot and divided by the number of plants.

c. **Anthesis date**: measured as the number of days after planting when 50% of the plants shed pollen.

d. **Silking date**: measured as the number of days after planting when 50% of the plants produced silks.

e. **Anthesis-silking intervals**: determined by measuring the number of days after planting when 50% of the plants shed pollen (anthesis date, AD) and extrude silks (silking date, SD) were recorded and ASI calculated as ASI = SD – AD.

f. **Ear position**: measured as the ratio of ear height to plant height. Small values indicate low ear position and large values will indicate high ear position.

g. **Ear height (cm)**: measured as height from ground level up to the base of the upper most cobs bearing internodes.

h. **Plant height (cm)**: measured as the distance between the base of a plant to the insertion point of the top tassel, measured after pants completely flowered since that's when they reach their maximum height.

i. **Root lodging**: measured as percentage of the plants per plot which have their stems inclining by more than 45^{o}.

j. **Stem lodging**: measured as the percentage of plants per plot that have their stems broken below the ear.

k. **Grain moisture**: measured as a percentage of water content of grain at harvest.

l. **Yield score**: rating on a 1-5 scale, where 1 has desirable ears and 5 poorest ears.

m. **Ear rot:** number of ears rotten per plot.

3.4.4 Data analysis

The data was analyzed using and GenStat 17th edition (Payne et al., 2011) and Path Analysis was done with SAS version 9.3 (SAS Institute Inc., 2012). Each phenotypic trait was analyzed using the model below in GenStat.

$$\mathbf{Y_{ijq} = U + r_i + b_{ij} + L_q + e_{ijq}}$$

Where, U = overall trial mean

r_i = effect of i^{th} replications

b_{ij} = effect of the j^{th} blocks within the i^{th} rep effects

L_q = effect of the inbred lines

e_{ijq} = random experimental error effects

3.4.5 Estimation of genetic parameters

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

Heritability

Heritability in a broad sense was estimated as the ratio of genotypic variance to the phenotypic variance and expressed in percentage (Hallauer and Miranda, 1988).

$$H^2 = \frac{\sigma^2 g}{\sigma^2 g + \sigma^2 e} * 100$$

Where, $\sigma^2 g$ = genotypic variance, $\sigma^2 e$ = error variance. $\sigma^2 p = (\sigma^2 g + \sigma^2 e)$ = phenotypic variance.

Genetic advance

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

$$GA = i\ \sigma p\ H^2$$

Where:

i = efficacy of selection (selection intensity) which is 2.06 at 5% proportion selected

σp = phenotypic standard deviation

H^2 = heritability in a broad sense

Genetic advance as per cent of mean

GA as per cent of mean = $\frac{GA}{X} * 100$

Where:

GA = genetic advance

X = general mean of character

Genotypic and phenotypic coefficient of variation

The genotypic and phenotypic coefficient of variation were calculated according to Burton and Devane (1953) as cited by (Darbeshwar, 2000) and expressed as percentage as follows.

Genotypic coefficient of variation: GCV = $\frac{\sqrt{\sigma^2 g}}{X} * 100$

Phenotypic coefficient of variation: PCV = $\frac{\sqrt{\sigma^2 p}}{X} * 100$

Where, $\sigma^2 g$ = Genotypic variation, $\sigma^2 p$ = Phenotypic variation and X = general mean of the character.

3.4.6 Regression and Path coefficient analysis

Both regression and phenotypic path analysis were performed using SAS version 9.3 (SAS Institute Inc., 2012). For regression the yield was used as the response variate and agronomic traits as independent variates. Path analysis was performed to determine the direct and indirect effects of the traits.

3.5 Genotyping

3.5.1 Greenhouse nursery

The 50 maize inbred families were planted in the tunnel at the University of KwaZulu-Natal (UKZN), Pietermaritzburg campus. Each family was replicated four times with three seeds planted in each pot. Pine bark medium was used as a growth media. Drip irrigation system was used, whereby each pot had one dripping head.

3.5.2 DNA sampling and isolation

DNA was extracted from the 50 inbred families that were planted in the tunnel at UKZN. Three leaf discs (punches) were harvested from two plants in each family at seven weeks after planting. They were put in a specific well position, each well representing an individual family. After all 50 wells were filled, each strip of a tube was sealed using perforated strip cap. The desiccant sachet was placed directly on top of the strip cap-sealed tubes and the plastic lid was replaced on top. The storage rack was secured by using an elastic band and was placed inside a sealable plastic bag. The sealed bag was placed into the plant kit box and then the samples were shipped to LGC Genomics Laboratory for genotyping in the United Kingdom.

3.5.3 SNP selection and amplification

In accordance with the protocol supplied by LGC genomics laboratory, Kompetitive Allele Specific Polymerase Chain Reaction (KASP) genotyping assays were used. These were based on competitive allele-specific PCR and enable bi-allelic scoring of Single Nucleotide Polymorphisms (SNPs) and insertion and deletions (Indels) at specific loci.

The SNP-specific KASP Assay mix and the universal KASP Master mix (supplied at 2X concentration) were used. KASP Master mix contains Taq polymerase enzyme and passive reference dye, 5-carboxy-X-rhodamine, succinimidyl ester (ROX) and $MgCl_2$ in an optimized buffer solution. The two mix were added to DNA samples then a thermal cycling was performed, followed by an end-point fluorescent read. Allele-specific primers each harbor a unique tall sequence that correspond with a universal fluorescence resonant energy transfer (FRET) cassette; one lebelled with FAM™ dye and the other with HEX™ dye. During thermal cycling, the relevant allele-specific primer would bind to the template and elongate, thus attaching the tail sequence to the newly synthesized strand. The complement of the allele-specific tail sequence was then generated during subsequent rounds of PCR, enabling the FRET cassette to bind to the DNA. Bi-allelic discrimination was achieved through the competitive binding of the two allele-specific forward primers. If the genotype at a given SNP was homozygous, only one of the two possible fluorescent signal was generated. If the genotype was heterozygous, a mixed fluorescent was generated (Figure 3.4). For the current study, a total of 391 SNP markers were used (Table 3.3 in the appendix section.)

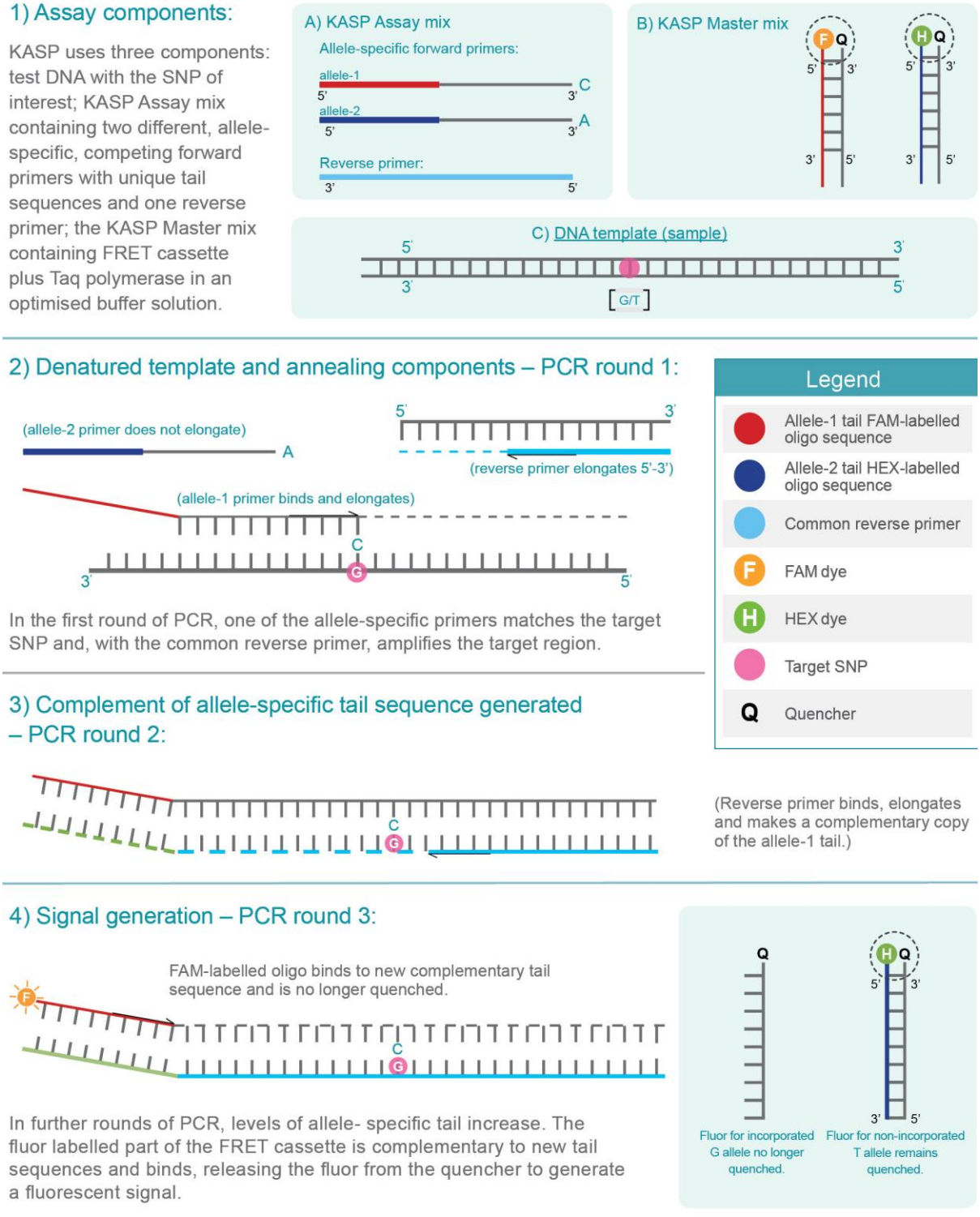


Figure 3.4: An overview of how SNP markers were selected and amplified through KASP (LGC genomics laboratory, 2016).

3.5.4 Data analysis

After completion of the initial 35 PCR cycles, all genotyping reaction plates were ran and read on a BMGPHERAStar plate reader. The plates were then recycled (3 cycles per recycle step) and read after each recycle step. Once the PCR had reached the endpoint that was the identification of plates being complete. After completion, an LGC in-house Kraken software automatically call genotypes for samples. The data read from Kraken was then accessed. For each SNP marker, number of samples genotyped as homozygous for allele X and allele Y, allele frequency, number of samples genotyped as heterozygous for allele X and Y were computed.

No template controls (NTCs) were included on each plate to enable the detection of contamination or non-specific amplification. The number of genotypes that were callable which had to be greater than 90% and minor allele frequency had to be greater than 2% unless the SNP was known to be of very low frequency. A chi-squared value (X^2) was also generated and it assessed distribution in multinomial datasets based on the Hardy-Weinberg equation. All these were also included on the analysis for quality control check on per SNP basis.

3.5.5 Genetic diversity analysis

Genotypic data were subjected to analyses with various measures of genetic diversity within and among genotypes using GenAlex software version 6.5 (Peakall and Smouse, 2007). Genetic diversity parameters such as total number of alleles per locus (N_a), number of effective alleles per locus (N_e), observed heterozygosity (H_o), Shannon's Information Index (I), gene diversity (H_e) and polymorphic information content (PIC) were determined using the protocol of Nei and Li (1979). To examine the degree of population differentiation, other genetic parameters such as differentiation (F_{ST}), were estimated using GenAlex.

3.5.6 Cluster analysis

The genotypic data were used to obtain a dissimilarity matrix using the Jaccard index. The matrix was used to run a cluster analysis. Cluster analysis was done based on neighbor-joining algorithm using the un-weighted pair group method using arithmetic average (UPGMA) in DARwin 5.0 software (Perrier and Jacquemoud-Collet, 2015). A dendrogram was then generated on the dissimilarity matrix. To investigate the genetic relationships among inbred families, genetic distances between all pairs of individual families were estimated to draw a dendrogram. Bootstrap analysis was performed for node construction using 10,000 bootstrap values.

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CHAPTER FOUR

RESULTS

4.1 Introduction

The two sites used in the study represent different environments; therefore the results are based on an individual site basis with the addition of the combined data. The genotypic results are also outlined under this section with 46 inbred families analyzed.

4.2 Phenotyping

4.2.1 Site performance

The analysis of variance (ANOVA) for Ukulinga data is presented in Table 4.1. The mean square for most traits were highly significant ($P<0.01$ and $P<0.001$). Root lodging, stem lodging, yield score and grain moisture content data were significant ($P<0.05$). Only ear rot data was not significant ($P>0.05$).

At Cedara (Table 4.2), the mean squares of silking date, anthesis date, anthesis-silking intervals, ear position were highly significant at $P<0.01$ and $P<0.001$. Grain yield, ear prolificacy, ear height, plant height, root lodging and grain moisture content were significant at $P<0.05$. Ear rot was again not significant ($P>0.05$) even at Cedara as it was at Ukulinga. Stem lodging and yield score were also not significant at Cedara.

The ANOVA of the combined sites data is presented in Table 4.3. Ten traits out of thirteen were highly significant ($P<0.01$ and $P<0.001$. Stem lodging was the only traits that was significant at $P<0.05$. The yield score and again ear rot were not significant ($P>0.05$). The interaction between sites and the families was highly significant ($P<0.001$) for anthesis-silking interval. Grain yield was significant ($P<0.05$) together with ear prolificacy, root lodging, grain moisture content and yield score.

Table 4.1: Analysis of Variance of 50 maize inbred families for quantitative traits at Ukulinga Research Farm.

Change	d.f	GY	EPP	AD	SD	ASI	EPO	EH	PH	RL	SL	GMC	YS	ER
Rep	1	1.33	0.09	6.25	6.76	0.01	0.003	86.50	59.30	78.23	207.81	6.76	0.36	18.49
Rep.Bloc	8	1.60	0.11	23.83	30.08	4.50	0.003	164.30	422.70	88.90	71.77	7.70	0.13	8.50
COV with No. Plants	1	5.98***	0.28**	52.41***	27.17*	4.11	0.004	166.30	7.50	8.89	1.03	0.43	0.05	10.06
Family	49	0.42***	0.11***	12.52***	14.86***	4.34**	0.006**	289.1***	375.90***	46.77*	122.12*	3.02*	0.15*	4.87
Residual	40	0.16	0.03	2.23	3.96	1.95	0.00	107.50	119.10	31.13	81.24	1.81	0.08	4.27
Mean		2.10	1.20	70.89	72.82	1.93	0.43	84.56	197.11	3.32	4.62	15.84	2.98	14.69
SE		0.40	0.17	1.49	1.99	1.40	0.05	10.37	10.91	5.58	9.01	1.35	0.29	2.07
LSD		0.81	0.35	3.06	4.08	2.89	0.11	21.27	22.30	11.44	18.49	2.76	0.58	4.24
CV%		18.84	14.37	2.11	2.73	72.32	12	12.26	5.54	167.86	194.89	8.49	9.56	79.18

*****, ******, ******* indicate data level of significant at 5%, 1% and 0.1% respectively.

COV = Covariate. GY = grain yield. EPP = ear prolificacy. AD = anthesis date. SD = silking date. ASI = anthesis-silking interval. EPO = ear position. EH = ear height. PH = plant height. RL = root lodging. SL = stem lodging. GMC = grain moisture content. YS = yield score. ER = ear rot

Table 4.2: Analysis of Variance of 50 maize inred families for quantitative traits at Cedara Research Station.

Source	d.f.	GY	EPP	AD	SD	ASI	EPO	EH	PH	RL	SL	GMC	YS	ER
Rep	1	0.35	0.07	40.81	19.26	2.04	0.00002	43.39	148.80	461.10	16.27	25.55	0.00	0.91
Rep.block	8	0.30	0.20	25.21	26.76	3.24	0.01	61.30	538.60	2206.60	5.00	8.63	1.01	0.83
COV with No. Plants	1	0.77**	0.01	1.60	7.05	3.17*	0.00002	53.27*	544.60	388.50	72.806**	0.02	1.60*	0.16
Family	49	0.15*	0.07*	14.18**	20.74***	3.51***	0.00444**	18.38*	396.7*	483*	6.90	8.65*	0.35	0.47
Residual	40	0.08	0.04	5.70	6.94	0.71	0.002	9.31	191.50	289.40	6.51	4.57	0.27	0.57
Mean		0.70	0.66	76.03	74.67	0.34	0.39	10.52	175.24	69.41	0.65	17.62	4.63	0.53
SE		0.28	0.19	2.39	2.63	0.84	0.04	10.04	13.84	17.01	2.55	2.14	0.52	0.75
LSD		0.58	0.40	4.98	5.44	1.74	0.09	6.30	28.30	34.79	5.22	4.41	1.07	1.56
CV%		39.60	29.23	3.15	3.46	337.78	11.15	29.16	7.90	24.51	390.50	12.13	11.21	149.16

*****, ******, ******* indicate level of significant of data at 5%, 1% and 0.1%, respectively.

COV = Covariate. GY = grain yield. EPP = ear prolificacy. AD = anthesis date. SD = silking date. ASI = anthesis-silking interval.

EPO = ear position. EH = ear height. PH = plant height. RL = root lodging. SL = stem lodging. GM = grain moisture content.

YS = yield score. ER = ear rot

Table 4.3: Analysis of Variance of 50 maize inbred families for quantitative traits across the two sites.

Source	df	GY	EPP	AD	SD	ASI	EPO	EH	PH	RL	SL	GMC	YS	ER
Site	1	98.02	14.59	1236.26	518.28	138.24	0.06	11858.00	23914.80	218376.70	788.58	157.12	134.83	220.43
Site.Rep	2	0.84	0.08	23.53	13.01	1.03	0.00	53.40	104.10	269.70	112.04	16.15	0.18	9.70
Site.Rep.Block	16	0.95	0.15	24.52	28.42	3.87	0.01	220.10	480.60	1147.70	38.39	8.16	0.57	4.67
COV with No. Plants	1	6.46***	0.25**	30.229**	9.38	7.062*	0.00	247.10	213.00	166.40	13.13	0.39	0.23	6.19
Family	49	0.39***	0.12***	23.323***	29.13***	3.805***	0.008***	454.2***	599.7***	291.60**	70.29*	7.03***	0.23	2.80
Site.Family	49	0.19*	0.053*	3.83	6.64	4.056***	0.0020	75.10	177.70	241.80*	56.14	4.62*	0.28*	2.51
Residual	81	0.12	0.03	3.89	5.35	1.34	0.0023	103.10	154.60	159.00	45.65	3.14	0.18	2.47
Mean		1.41	0.93	73.37	74.42	1.17	0.41	76.87	186.17	36.37	2.64	16.71	3.79	1.57
SE		0.34	0.18	1.97	2.31	1.16	0.05	10.15	12.43	12.61	6.76	1.77	0.42	1.57
LSD		0.49	0.26	2.82	3.34	1.67	0.07	14.41	17.65	17.89	9.59	2.53	0.60	2.24
CV%		24.32	19.61	2.69	3.11	104.48	11.55	13.21	6.68	34.67	256.01	10.60	11.14	100.64

*****, ******, ******* indicate level of significant at 5%, 1% and 0.1% respectively.

COV = Covariate. GY = grain yield. EPP = ear prolificacy. AD = anthesis date. SD = silking date. ASI = anthesis-silking interval. EPO = ear position. EH = ear height. PH = plant height. RL = root lodging. SL = stem lodging. GM = grain moisture content. YS = yield score. ER = ear rot.

4.2.2 Mean performance

The top ten inbred families that displayed the highest yield at Ukulinga are presented in Table 4.4: and the data ranged from 2.42 to 3.57 t ha^{-1} while yield data for the bottom ten ranged from 1.10 to 1.69 t ha^{-1}. The average yield of the 50 families was 2.10 t ha^{-1}. At Cedara the top ten inbred families ranged from 0.87 to 1.59 t ha^{-1} (Table 4.5), whereas the bottom ten ranged from 0.06 to 0.48 t ha^{-1}. The average yield at Cedara was 0.70 t ha^{-1}. The

Table 4.6: shows the mean of the families yield across the two sites that performed well together with their performance on each site. The mean yield at both site was 1.41 t ha^{-1}.

Looking at the average rank for earliness among the high yielding families at Ukulinga, only one inbred family (15MAK8_74) among the top ten families could be considered early maturing. All the other families were late maturing inbred families. At Cedara none of the top yielding line was characterized as being early maturing (Table 4.5). Across both sites among the top best yielding families, one line fell under the top ten early maturing line (Table 4.6).

Early maturing families are presented by the average rank which include anthesis date, silking date and grain moisture content. At Ukulinga and across the two sites, 23 families were early (Table 4.8 and Table 4.10) in the appendix. Cedara had 24 families that matured early (Table 4.9). Table 4.7 only displays the top ten families for the two sites and across both site that were early maturing. Five of the families were early on both sites and also across both sites. The families' earliness were compared with their yield rank and only one line was both early maturing and high yielding.

Table 4.4: The grain yield mean of the top ten maize inbred familes together with earliness rank at the Ukulinga Research Farm.

					Earliness rank				
	Grain yield		Anthesis date		Silking date		Grain moisture content		
Inbred	Mean (t ha^{-1})	Rank	Mean (days)	Rank	Mean (days)	Rank	Mean (%)	Rank	Average rank for earliness
15MAK8_75	3.57	1	74.79	46	76.33	46	18.89	49	47
15MAK9_5	3.22	2	70.95	28	71.47	18	15.23	21	22
15MAK9_10	2.99	3	70.78	27	73.79	32	16.28	35	31
15MAK9_12	2.89	4	68.45	9	69.97	10	15.23	22	14
15MAK8_104	2.86	5	69.61	20	70.11	11	15.52	25	19
15MAK8_57	2.86	6	71.45	30	73.47	30	15.73	28	29
15MAK8_74	2.68	7	66.78	1	67.79	2	14.88	12	5
15MAK8_90	2.54	8	79.75	50	80.21	50	20.00	50	50
15MAK8_84	2.48	9	71.75	33	72.21	21	16.20	34	29
15MAK8_19	2.42	10	69.45	19	70.47	14	14.18	5	13
SE	0.40		1.49		1.99		1.35		
SED	0.63		1.22		1.41		1.16		

Table 4.5: The grain yield mean of the top ten maize inbred families together with their earliness rank at the Cedara Research Station.

					Earliness rank				
	Grain yield		Anthesis date		Silking date		Grain moisture content		
Inbred	Mean (t ha^{-1})	Rank	Mean (days)	Rank	Mean (days)	Rank	Mean (%)	Rank	Average rank for earliness
15MAK8_57	1.59	1	77.28	38	76.30	24	20.220	48	37
15MAK8_90	1.54	2	82.31	49	81.30	47	20.070	46	47
15MAK8_125	1.24	3	76.50	27	75.46	19	18.740	35	27
15MAK8_144	1.15	4	83.73	50	82.74	49	18.500	33	44
15MAK8_74	1.12	5	70.11	2	69.11	3	18.530	34	13
15MAK8_75	0.99	6	80.04	45	79.00	42	17.260	22	36
15MAK8_31	0.91	7	74.51	15	73.56	13	18.440	31	20
15MAK8_91	0.89	8	78.14	43	77.11	31	18.270	29	34
15MAK8_89	0.88	9	77.91	41	81.48	48	19.820	44	44
15MAK8_76	0.87	10	77.68	40	77.61	36	19.270	39	38
SE	0.28		2.39		2.63		2.14		
SED	0.23		1.55		1.62		1.46		

Table 4.6: The grain yield mean of the top ten maize inbred families together with earliness rank across the two sites.

									Earliness rank		
	Grain yield				Anthesis date		Silking date		Grain moisture content		
Inbred	Mean (t ha^{-1})	Rank	Ukulinga rank	Cedara rank	Mean (days)	Rank	Mean (days)	Rank	Mean (%)	Rank	Average rank for earliness
15MAK8_75	2.31	1	1	6	77.40	48	77.62	45	18.12	43	45
15MAK8_57	2.21	2	6	1	74.39	33	74.92	26	17.92	38	32
15MAK9_5	2.03	3	2	23	73.78	28	73.94	22	16.66	27	26
15MAK8_90	2.02	4	8	2	81.16	50	80.90	50	19.91	50	50
15MAK8_74	1.91	5	7	5	68.44	1	68.46	1	16.68	28	10
15MAK9_10	1.88	6	3	18	74.00	31	75.02	28	16.52	22	27
15MAK8_104	1.79	7	5	25	71.64	14	71.40	10	16.35	19	14
15MAK8_144	1.76	8	17	4	78.64	49	80.15	49	18.01	39	46
15MAK9_12	1.73	9	4	37	72.12	18	73.35	17	16.01	14	16
15MAK8_89	1.66	10	13	9	75.60	40	78.04	46	18.82	48	45
SE	0.34				1.97		2.31		1.77		
SED	0.58				1.40		1.52		1.33		

Table 4.7: Top ten early maturing mazie inbred families rank for each site and across both sites together with grain yield rank.

	Ukulinga			Cedara			Both sites		
Number	Inbred	Average rank	Grain yield rank	Inbred	Average rank	Grain yield rank	inbred	Average rank	Grain yield rank
1	15MAK8_1	3	34	15MAK8_59	4	43	15MAK8-17	5	20
2	15MAK8_74	5	7	15MAK8_14	7	42	15MAK8-59	5	22
3	15MAK9_16	6	21	15MAK8_17	7	22	15MAK8-14	6	41
4	15MAK8_31	8	15	15MAK8_4	8	41	15MAK8-99	7	12
5	15MAK8_99	9	14	15MAK8_99	9	12	15MAK9-16	7	18
6	15MAK8_59	9	16	15MAK9_16	10	20	15MAK8-4	8	14
7	15MAK8_11	10	19	15MAK8_135	10	16	15MAK8-135	9	42
8	15MAK8_130	10	12	15MAK8_32	13	21	15MAK8-74	10	5
9	15MAK8_14	10	35	15MAK8_74	13	5	15MAK8-32	10	37
10	15MAK8_135	11	44	15MAK8_5	13	34	15MAK8-130	12	17

4.2.3 Heritability and genetic advance

The heritability percentage was characterized according to Robinson et al. (1949), whereby 0-30% is low heritability, 30-60% is moderate heritability and >60% is high heritability.

In the study, the heritability ranged from low to high for the different traits (Table 4.11). The highest estimate heritability computed was for anthesis date only whereas grain yield, ear prolificacy, silking date, anthesis-silking interval, ear position, ear height and plant height had moderate heritability value. Root lodging, stem lodging, grain moisture, yield score and ear rot had low heritability estimates.

At Cedara (Table 4.12), anthesis-silking interval had the high heritability estimate of 66.23%. Traits that exhibited moderate heritability includes grain yield, ear prolificacy, anthesis date, silking date, ear position, ear height, plant height and grain moisture content. Root lodging stem lodging, yield score and ear rot had low heritability estimates. When looking at the combined data, none of the traits exhibited high heritability.

Genetic advance range at Ukulinga was from 0.05 to16.81, with plant height exhibiting the highest genetic advance of 16.81 and ear position having the lowest genetic advance of 0.05 (Table 4.11). At Cedara it ranged from 0.05-12.32. Plant height was also the traits with high genetic advance and ear position with the lowest one.

4.2.4 Coefficient of variation

Phenotypic coefficient of variation was higher than genotypic coefficient of variation for all traits at both sites but for the combined data it was the genotypic coefficient of variation that was higher for all traits when compared to phenotypic coefficient of variation. Genotypic coefficient of variation at Ukulinga ranged from 3.21 to 97.76 (Table 4.11) and 0.00 to 351.92 (Table 4.12) at Cedara. Phenotypic coefficient of variation ranged from 3.83 to 218.03 at Ukulinga and at Cedara it ranged from 4.15 to 432.43. For the combined data, genotypic

coefficient of variation ranged from 2.93 to 94.04 and phenotypic coefficient of variation ranged from 0.95 to 44.40.

Table 4.11: Heritability estimates for quantitative traits of maize inbred families at Ukulinga Research Farm.

Trait	σ^2g	Heritability (H^2) %	GA	GCV %	PCV %
Grain yield	0.13	45.78	0.51	17.31	25.59
Ear prolificacy	0.04	56.53	0.30	16.39	21.80
Anthesis date	5.14	69.75	3.90	3.20	3.83
Silking date	5.45	57.94	3.66	3.21	4.21
Anthesis silking interval	1.19	38.00	1.39	56.61	91.84
Ear position	0.00	36.13	0.05	9.02	15.01
Ear Height	90.80	45.79	13.28	11.27	16.65
Plant height	128.40	51.88	16.81	5.75	7.98
Root lodging	7.82	20.08	2.58	84.14	187.78
Stem lodging	20.44	20.10	4.18	97.76	218.03
Grain moisture	0.61	25.09	0.80	4.92	9.81
Yield score	0.03	29.49	0.21	6.18	11.39
Ear rot	0.30	6.50	0.29	20.88	81.89

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

Table 4.12: Heritability estimates for quantitative traits of maize inbred families at Cedara Research Station.

Trait	σ^2g	Heritability (H^2) %	GA	GCV %	PCV %
Grain yield	0.04	31.60	0.22	26.92	47.89
Ear prolificacy	0.02	30.89	0.15	19.44	34.97
Anthesis date	4.24	42.65	2.77	2.71	4.15
Silking date	6.90	49.85	3.82	3.52	4.98
Anthesis silking interval	1.40	66.23	1.98	351.92	432.43
Ear position	0.00	39.53	0.05	9.01	14.33
Ear Height	4.53	32.73	2.51	20.23	35.36
Plant height	102.60	34.89	12.32	5.78	9.79
Root lodging	96.80	25.06	10.15	14.17	28.31
Stem lodging	0.19	2.86	0.15	67.06	396.21
Grain moisture	2.04	30.89	1.64	8.11	14.59
Yield score	0.04	12.48	0.14	4.23	11.97
Ear rot	-0.05	-9.69	-0.14	0.00	135.15

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

4.2.5 The frequency distribution of families for selected traits

Three traits (grain moisture content, silking date and anthesis date) were an indication of earliness including grain yield and ear prolificacy were selected to observe their frequency distribution.

Grain yield showed continuous distribution in both environments. At Ukulinga it was skewed to the right with very few lines that yielded above 2.5 t ha^{-1}. A lot of lines yielded between 1.6 and 2.4 t ha^{-1} (Fugure 4.1). At Cedara the yield was normally distributed with many lines yielding between 0.4 and 0.9 t/ha. Anthesis date was normally distributed at Ukulinga and skewed to the right at Cedara as many families were late (75 - 77 days) whereas at Ukulinga many families flowered early (69 - 71 days).

With respect to silking date, many inbred families were early at Ukulinga. They ranged between 69 and 74 days (Figure 4.3) while many lines at Cedara only started silking between 78 and 90 days. Adding on, grain moisture showed continuous distribution at Ukulinga and at Cedara it was more distributed to the right, a lot of inbred families having high grain moisture content. Ear prolificacy was normally distributed at Cedara. There were no lines with more than one ears. At Ukulinga, the highest number of ears was two and few of the lines had two ears (Figure 4.5).

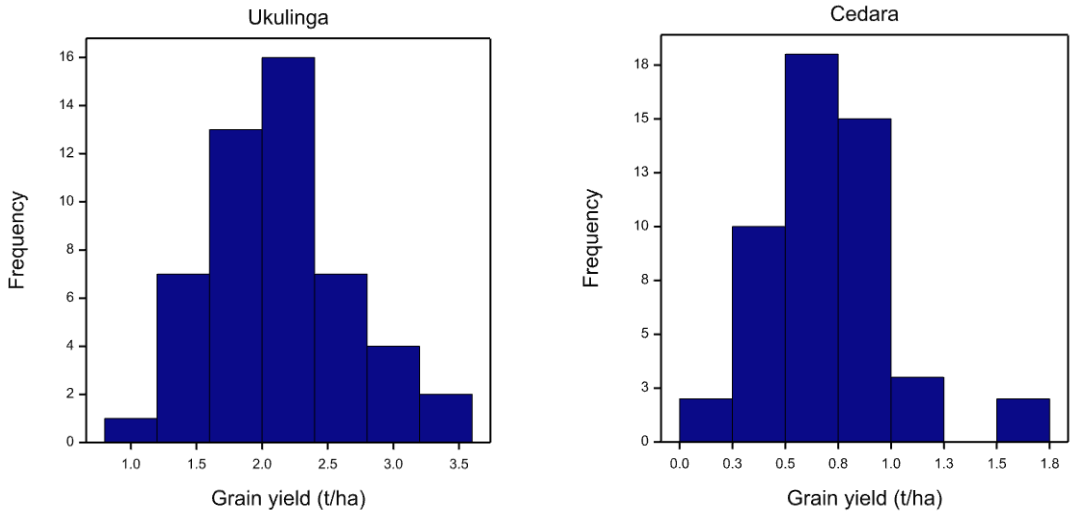

Figure 4.1: The histogram of grain yield of the inbred families for the two sites.

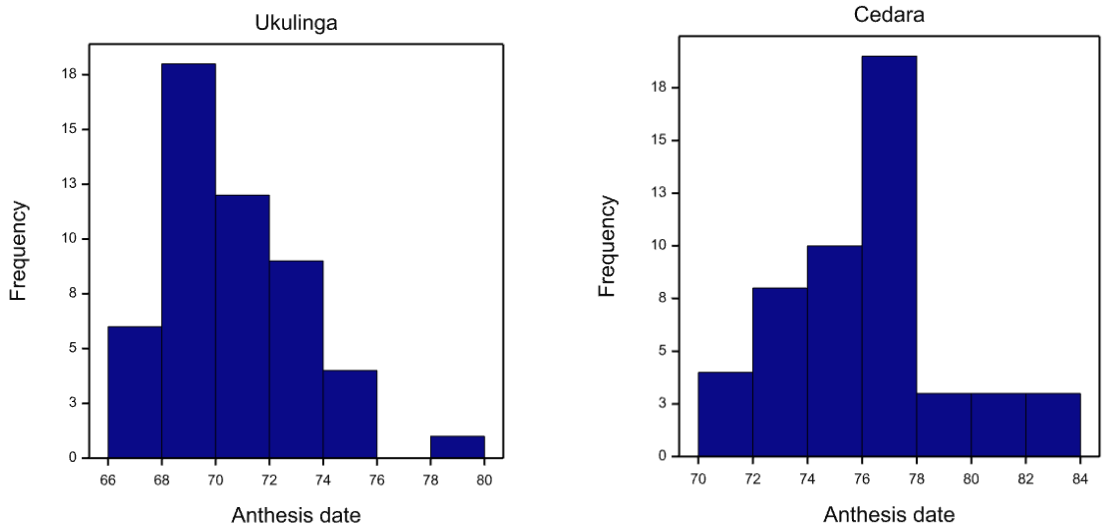

Figure 4.2: The histogram for anthesis date for the inbred families for the two sites.

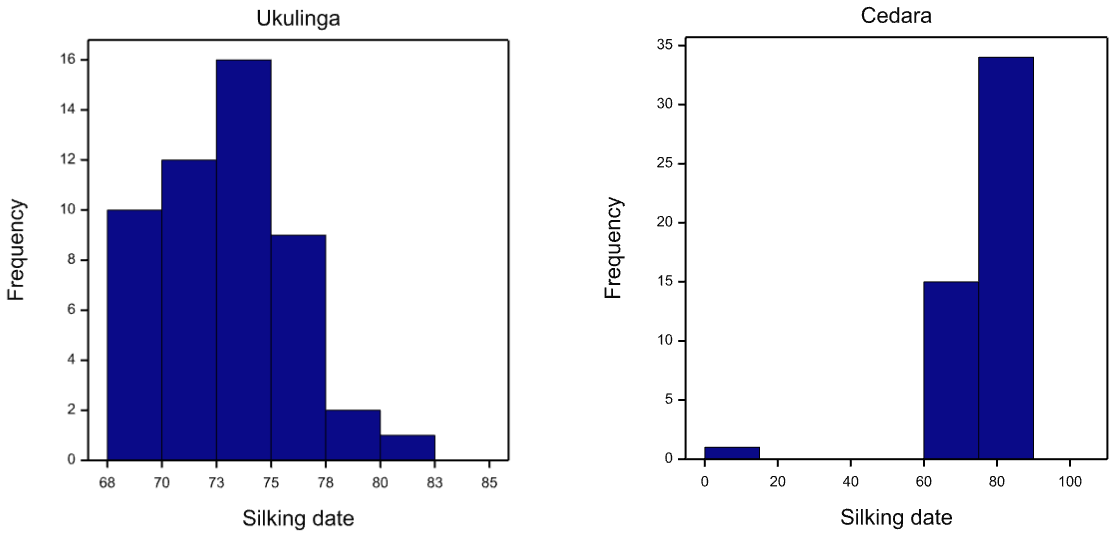

Figure 4.3: The histogram for silking date of the inbred families for the two sites.

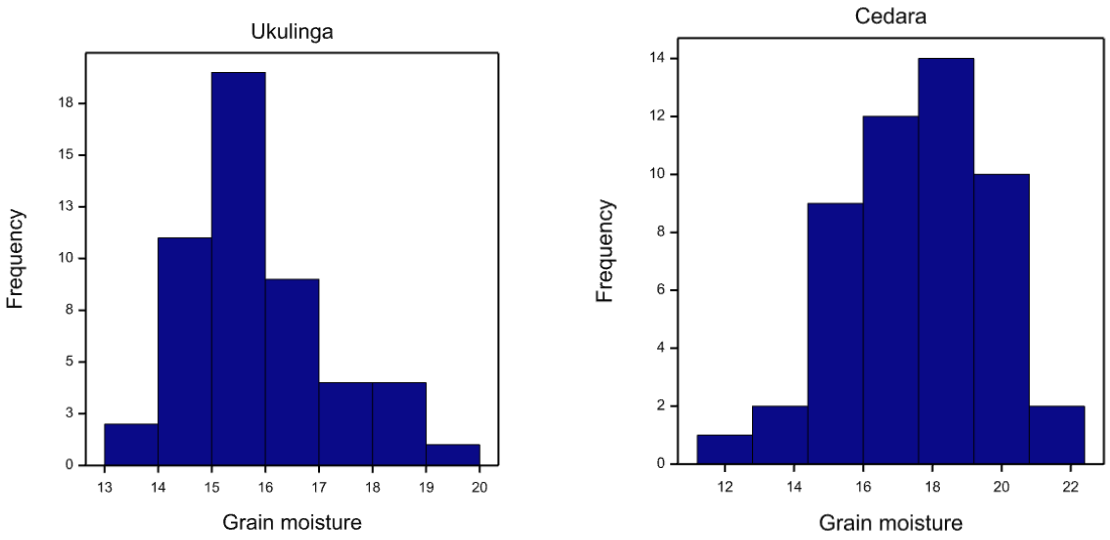

Figure 4.4: The histogram for grain moisture content of the inbred families for the two sites.

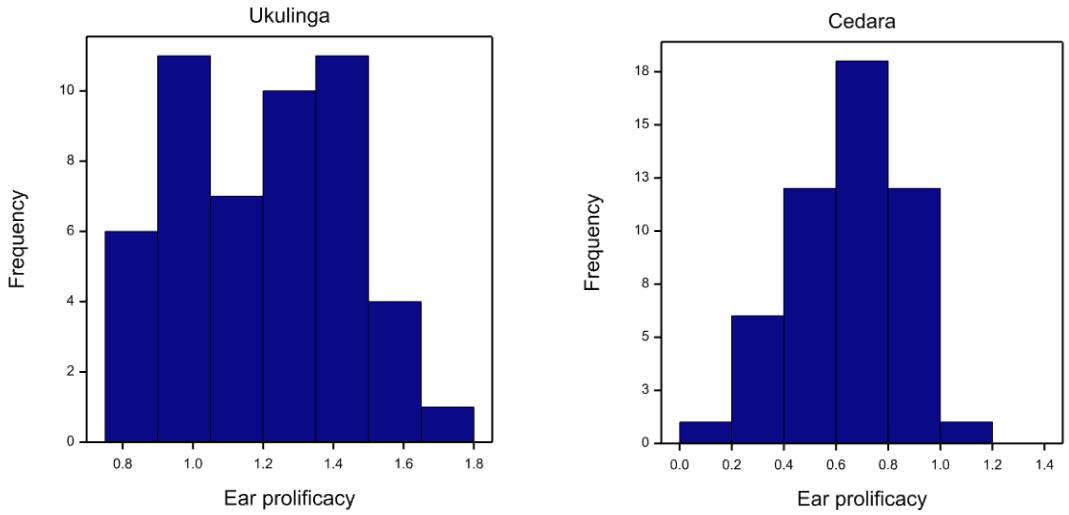

Figure 4.5: The histogram for ear prolificacy of the inbred families for the two sites.

4.2.6 The relationship between phenotypic traits

The two environments that were used represented two different environments. Therefore correlation analysis was conducted between traits for each environment (Table 4.13 and 4.14). The study reveals that there were significant correlations between grain yield and secondary traits and among the independent secondary traits at both site.

At Ukulinga the grain yield was highly correlated ($P<0.01$ and $P<0.001$) and positively correlated with ear height and ear prolificacy (Table 4.13). It was also highly significant ($P<0.01$) but negatively correlated with anthesis-silking interval and yield score whereas at Cedara, anthesis-silking interval was only significant ($P<0.05$) but also negatively correlated with grain yield. Grain yield at Cedara was also highly significant ($P<0.001$) and positively correlated with ear height and ear prolificacy but this time with also grain moisture content. The same highly significant data with negative correlation of grain yield and yield score was also observed at Cedara (Table 4.14). Grain yield was significant ($P<0.05$) and positively correlated with ear position and plant height at both sites.

Grain moisture content at Cedara was significant ($P<0.05$) and positively correlated with a lot of traits which includes ear prolificacy, anthesis date, silking date, anthesis-silking interval, ear position, ear height and plant height. The yield score was the only one that was significant but with a negative correlation with yield at Cedara. At Ukulinga, grain moisture content was highly significant ($P<0.001$) and positively correlated with anthesis date and silking date and was significant ($P<0.05$) and positively correlated with ear position, ear height and stem lodging.

Table 4.13: Phenotypic relationships of grain yield and secondary traits at the Ukulinga Research Farm

Traits	Grain yield (GY)	Ear prolificacy (EPP)	Anthesis date (AD)	Silking date (SD)	Anthesis silking interval (ASI)	Ear position (EPO)	Ear Height (EH)	Plant height (PH)	Root lodging (RL)	Stem lodging (SL)	Grain moisture content (GMC)	Yield score (YS)	Ear rot (ER)	Number of Plants (NP)
GY	-													
EPP	0.68***	-												
AD	0.08	0.05	-											
SD	-0.16	-0.27*	0.85***	-										
ASI	-0.44**	-0.59***	-0.13	0.42**	-									
EPO	0.30*	0.19	0.42**	0.24*	-0.28*	-								
EH	0.44**	0.20	0.49***	0.29*	-0.30*	0.84***	-							
PH	0.32*	0.06	0.22	0.13	-0.12	-0.03	0.51***	-						
RL	0.05	0.06	0.35*	0.30*	-0.04	0.40**	0.44**	0.17	-					
SL	-0.09	0.15	-0.01	-0.11	-0.19	0.20	0.13	-0.09	-0.04	-				
GM	0.16	0.04	0.55***	0.54***	0.07	0.34*	0.34*	0.08	0.16	0.24*	-			
YS	-0.39**	-0.03	-0.07	-0.09	-0.05	-0.06	-0.14	-0.17	0.07	0.11	-0.13	-		
ER	-0.22	-0.10	0.19	0.34*	0.32*	-0.19	-0.18	-0.05	0.12	-0.03	0.33*	0.01	-	
NP	0.16	-0.14	-0.13	-0.03	0.17	-0.02	0.04	0.14	0.01	-0.43**	-0.25*	-0.12	-0.23	-

*****, ******, ******* indicate level of significant at 5%, 1% and 0.1% respectively

Table 4.14: Phenotypic reslationship of grain yield and secondary traits at the Cedara Research Station.

Traits	Grain yield (GY)	Ear prolificacy (EPP)	Anthesis date (AD)	Silking date (SD)	Anthesis silking interval (ASI)	Ear position (EPO)	Ear Height (EH)	Plant height (PH)	Root lodging (RL)	Stem lodging (SL)	Grain moisture content (GMC)	Yield score (YS)	Ear rot (ER)	Number of plants (NP)
GY	-													
EPP	0.78***	-												
AD	0.17	-0.21	-											
SD	0.23	0.16	0.01	-										
ASI	-0.29*	-0.37**	0.27*	0.24*	-									
EPO	0.27*	0.03	0.42**	0.15	0.12	-								
EH	0.80***	1.00***	-0.21	0.15	-0.37**	0.04	-							
PH	0.24*	-0.03	0.37**	0.03	0.04	0.24	-0.03	-						
RL	-0.02	0.03	0.14	-0.01	0.10	0.27*	0.04	-0.15	-					
SL	0.00	-0.09	-0.04	0.03	-0.06	0.00	-0.06	-0.18	-0.07	-				
GM	0.51***	0.30*	0.29*	0.24*	0.32*	0.30*	0.31*	0.26*	0.02	-0.13	-			
YS	-0.59***	-0.27*	-0.36*	-0.20	0.12	-0.24*	-0.27*	-0.39**	0.22	0.01	-0.34*	-		
ER	0.04	0.15	-0.08	-0.03	0.00	-0.18	0.16	-0.12	-0.15	-0.06	-0.14	0.02	-	
NP	0.10	-0.03	0.17	0.05	0.15	-0.07	-0.01	0.00	0.03	0.07	0.08	-0.01	0.06	-

*****, ******, ******* indicate level of significant at 5%, 1% and 0.1% respectively.

4.2.7 Path coefficient analysis

Regression data showing levels of significance for direct effects for Ukulinga and Cedara are presented in Table 4.15 and Table 4.16, respectively. At Ukulinga, silking date and grain moisture data were significant at 5% for regression of yield and yield score was highly significant (P<0.0001). Regression of yield on grain moisture was significant at 1% and yield score was highly significant (P<0.0001) at Cedara.

Ukulinga data for path analysis are presented in Table 4.17. Yield score was significant at P ≤ 0.001 while silking date and grain moisture content were significant at P ≤ 0.05. Ear height had the highest direct, positive effect on grain yield followed by number of plants, grain moisture, anthesis date, ear prolificacy, root lodging and plant height. While the yield score had the highest, negative direct effect on grain yield followed by silking date, stem lodging, ear position and ear rot. Ear position through ear height had the highest indirect, positive effect on grain yield followed by plant height through ear height, anthesis via ear height, ear prolificacy through silking date, number of plants through silking date and silking date via grain moisture. Anthesis date displayed the highest indirect, negative effect on grain yield through silking date followed by grain moisture through stem lodging and anthesis silking interval through ear height.

At Cedara, yield score was significant at P ≤ 0.001 and grain moisture content was significant at P ≤ 0.01. Ear height had the highest, positive direct effects on grain yield followed by grain moisture, anthesis date and silking date (Table 4.18). Ear prolificacy had the highest, negative direct effects on yield followed by ear position, yield score, plant height, number of plants, silking date and ear rot. For an indirect effect on grain yield, ear position had the highest, positive effect through ear height followed by plant height via ear height, anthesis silking date via ear prolificacy, silking date and yield score were equal through ear prolificacy and silking date through ear height. Grain moisture through ear prolificacy and yield score through ear height had the highest indirect and negative effects on grain yield followed by ear height via plant height and silking date and anthesis date through ear position.

Table 4.15: Regression data for the direct effects of secondary traits at Ukulinga.

Traits	Parameter Estimate	Standard Error	t Value	Pr > |t|
Ear prolificacy	0.05864	0.22042	0.27	0.7908
Anthesis date	0.0541	0.12438	0.43	0.6647
Silking date	-0.21892	0.12747	-1.72	0.0895*
Anthesis silking interval	0	.	.	.
Ear position	-0.08441	0.46863	-0.18	0.8575
Ear height	0.23722	0.53332	0.44	0.6576
Plant height	0.02633	0.29955	0.09	0.9302
Root lodging	0.04072	0.05676	0.72	0.475
Stem lodging	-0.08546	0.05617	-1.52	0.1319
Grain moisture	0.1323	0.06722	1.97	0.0523*
Yield score	-0.26159	0.0565	-4.63	<.0001***
Ear rot	-0.0489	0.06037	-0.81	0.4202
Number of plants	0.17364	0.1997	0.87	0.387

Table 4.16: Regression data for direct effects of secondary traits at Cedara.

Traits	Parameter Estimate	Standard Error	t Value	Pr > |t|
Ear prolificacy	-0.63499	0.55882	-1.14	0.2592
Anthesis date	0.13264	0.11758	1.13	0.2626
Silking date	-0.04768	0.1209	-0.39	0.6943
Anthesis silking interval	0	.	.	.
Ear position	-0.27893	0.46686	-0.6	0.5519
Ear height	0.45069	0.61008	0.74	0.4622
Plant height	-0.17913	0.31642	-0.57	0.5729
Root lodging	0.00166	0.04699	0.04	0.9719
Stem lodging	0.01242	0.04878	0.25	0.7997
Grain moisture	0.14213	0.05059	2.81	0.0062**
Yield score	-0.25765	0.05162	-4.99	<.0001***
Ear rot	-0.0077	0.04251	-0.18	0.8568
Number of plants	-0.06373	0.14282	-0.45	0.6566

Table 4.17: Direct (underlined and bold) and indirect effects of the secondary traits on 50 maize inbred families at Ukulinga. (R^2=0.75; Pr>F=<.0001)

Grain yield component	Ear prolificacy (EPP)	Anthesis date (AD)	Silking date (SD)	Anthesis silking interval	Ear position (EPP)	Ear height (EH)	Plant height (PH)	Root lodging (RL)	Stem lodging (SL)	Grain moisture content (GMC)	Yield score (YS)	Ear rot (ER)	Number of plants (NP)	Total correlation to grain yield
EPP	**0.04**	-0.01	0.09	0	-0.01	0.05	0	0	0	0	0.01	0	0.1	**0.44**
AD	0	**0.05**	-0.19	0	-0.03	0.09	0	0.01	0	0.05	0.02	0	-0.06	**-0.19**
SD	-0.01	0.05	**-0.22***	0	-0.02	0.04	0	0.01	0.01	0.06	0.03	-0.01	-0.06	**-0.35**
ASI	-0.03	-0.01	-0.1	**0**	0.02	-0.07	-0.01	0	0.01	0.02	0.02	-0.01	0	**-0.34**
EPO	0	0.02	-0.05	0	**-0.08**	0.2	0	0.01	-0.01	0.04	0	0.01	0.01	**0.2**
EH	0.01	0.02	-0.04	0	-0.07	**0.24**	0.01	0.01	0	0.03	0.02	0.01	0.02	**0.35**
PH	0.01	0	0.01	0	0.01	0.12	**0.03**	0	0.01	-0.01	0.03	0	0.02	**0.31**
RL	0	0.01	-0.04	0	-0.01	0.03	0	**0.04**	0	0.03	-0.02	0	-0.02	**0**
SL	0	0	0.02	0	-0.01	0.01	0	0	**-0.09**	0.02	-0.01	0	0	**-0.03**
GM	0	0.02	-0.09	0	-0.02	0.05	0	0.01	-0.01	**0.13***	0.03	-0.01	0	**0.09**
YS	0	0	0.02	0	0	-0.01	0	0	0	-0.02	**-0.26*****	0	0	**-0.3**
ER	-0.01	0	-0.03	0	0.01	-0.02	0	0	0	0.03	-0.02	**-0.05**	0.04	**-0.02**
NP	-0.01	-0.02	0.07	0	-0.01	0.03	0	0	0	0	0.01	-0.01	**0.17**	**0.52**

Table 4.18: Direct (underlined and bold) and indirect effects of the secondary traits on 50 maize inbred families at Cedara. (R^2=0.86; Pr>F=<.0001)

Grain yield component	Ear prolificacy (EPP)	Anthesis date (AD)	Silking date (SD)	Anthesis silking interval (ASI)	Ear position (EPP)	Ear height (EH)	Plant height (PH)	Root lodging (RL)	Stem lodging (SL)	Grain moisture content (GMC)	Yield score (YS)	Ear rot (ER)	Number of plants (NP)	Total correlation to grain yield
EPP	**-0.63**	-0.03	0.01	0	-0.02	0.04	-0.01	0	0	0.04	0.08	0	-0.01	0.77
AD	0.14	**0.13**	-0.04	0	-0.1	0.18	-0.05	0	0	0.02	0.05	0	0	0.05
SD	0.19	0.12	**-0.05**	0	-0.1	0.16	-0.04	0	0	0.03	0.03	0	0	-0.03
ASI	0.21	0.02	-0.02	**0**	-0.02	0.02	0	0	0	0.02	-0.03	0	-0.01	-0.22
EPO	-0.04	0.05	-0.02	0	**-0.28**	0.39	-0.03	0	0	0.02	0.06	0	0	0.25
EH	-0.06	0.05	-0.02	0	-0.24	**0.45**	-0.12	0	0	0.04	0.1	0	0	0.34
PH	-0.04	0.03	-0.01	0	-0.05	0.3	**-0.18**	0	0	0.04	0.09	0	-0.01	0.28
RL	0.07	0.01	0	0	-0.05	0.01	0.03	**0**	0	-0.01	-0.06	0	0.01	-0.15
SL	0.05	0	0	0	-0.01	-0.01	0.02	0	**0.01**	-0.02	0.01	0	0.02	-0.09
GM	-0.18	0.02	-0.01	0	-0.05	0.12	-0.05	0	0	**0.14****	0.06	0	-0.01	0.45
YS	0.19	-0.03	0.01	0	0.07	-0.18	0.07	0	0	-0.03	**-0.26****	0	0.01	-0.6
ER	-0.05	-0.01	0	0	0.02	-0.03	0	0	0	-0.01	0.02	**-0.01**	0	0.05
NP	-0.06	0	0	0	0	0.02	-0.01	0	0	0.02	0.05	0	**-0.06**	0.36

4.3 Genotyping

4.3.1 Marker characterization and genetic diversity among families

The statistics of genetic diversity parameters within and among genotypes are given in Table 4.19. The number of alleles and their frequency at each locus were analyzed as an indicator of polymorphism. A total of 391 alleles were observed in the 50 inbred families when characterized according to the chromosome number where markers were amplified. The mean number of alleles per locus for all the families was 39.1. The effective numbers of alleles (N_e) for all the loci were less than 2, with a mean of 1.93 alleles per locus. The polymorphic information content (PIC) for the SNP locus ranged from 0.42 to 0.51 (Figure 4.6), giving an average of 0.48. The mean gene diversity (H_e) was observed to be 0.49, with maximum and minimum values recorded by SNP markers 0.43 and 0.50 (Figure 4.7). The observed gene diversity within genotype ranged from 0.12 to 0.26 with the mean value of 0.18 for all the loci. The inbreeding coefficient ranged from 0.45 to 0.74.The DNA bases adenine to thymine (A/T) content mean is lower than that of guanine to cytosine (G/C) content but their standard deviation is equal (0.03).

Table 4.19: Genetic diversity between and among 46 maize inbred families s based on 391 SNP markers.

Chromosome	Genetic parameter							
	N_a	N_e	H_o	H_e	F_{IS}	PIC	A/T content	G/C content
1	53.0	1.96	0.24	0.50	0.51	0.49	0.51	0.49
2	41.0	1.87	0.13	0.47	0.73	0.46	0.62	0.38
3	41.0	1.78	0.12	0.43	0.74	0.42	0.50	0.50
4	40.0	1.89	0.26	0.48	0.45	0.47	0.31	0.69
5	40.0	1.97	0.14	0.50	0.70	0.49	0.55	0.45
6	40.0	1.98	0.13	0.50	0.74	0.50	0.48	0.52
7	34.0	1.96	0.20	0.50	0.60	0.51	0.41	0.59
8	37.0	1.96	0.14	0.50	0.71	0.49	0.39	0.61
9	33.0	1.89	0.16	0.48	0.67	0.47	0.50	0.50
10	32.0	1.99	0.21	0.50	0.59	0.50	0.59	0.41
Overall mean	39.1	1.93	0.18	0.49	0.63	0.48	0.48	0.52
SE	1.9	0.02	0.02	0.01	0.03	0.01	0.03	0.03

N_a= total number of alleles per locus; N_e= number of effective alleles per locus; H_o= observed gene diversity within genotypes; H_e= average gene diversity within genotypes; F_{IS}

= inbreeding coefficient; PIC= polymorphic information content; A= major allele frequency; SE= Standard error.

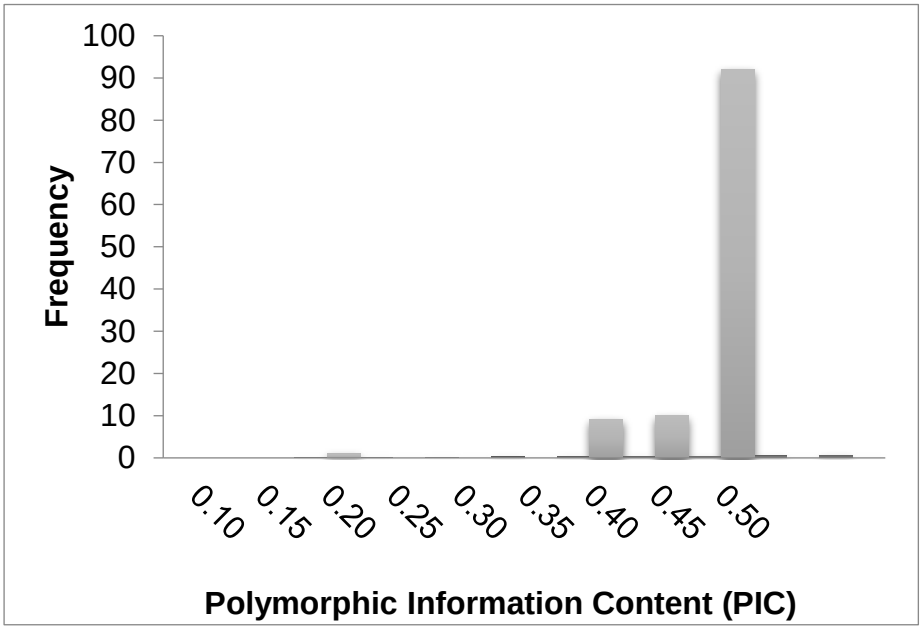

Figure 4.6: Frequency distribution on 391 SNP markers for PIC.

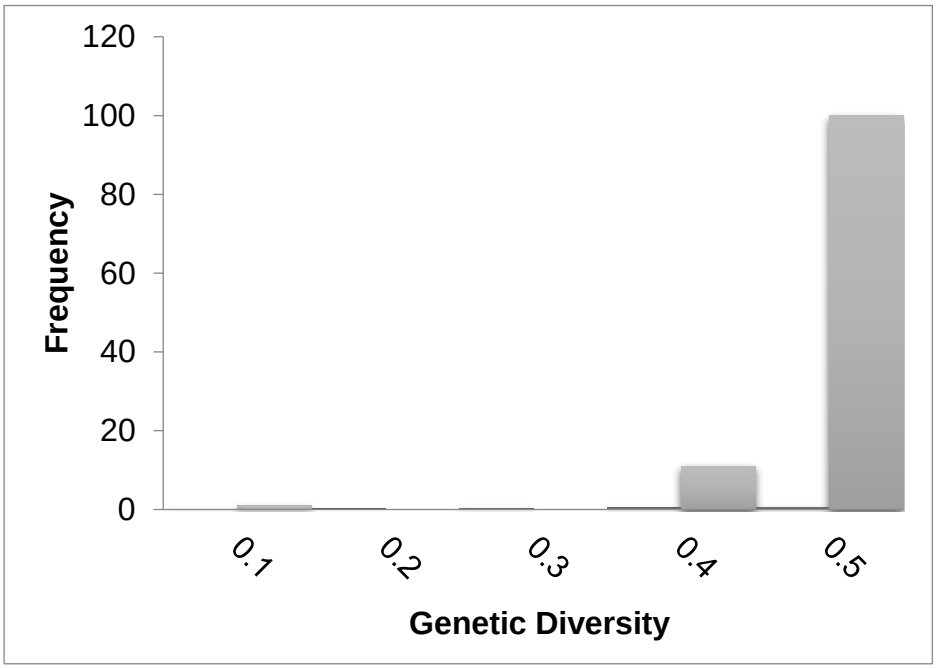

Figure 4.7: Frequency distribution of 391 SNP markers for genetic diversity.

4.3.2 Genetic distance

The SNP data revealed high diversity among the 46 maize inbred families. The lowest genetic distance was 0.15 and the highest was 0.56 as shown in Figure 4.8. The inbred families 15MAK8_90 / 15MAK8_35 were highly similar with 0.15 distance between them and 15MAK9_5 / 5MAK8_7, 15MAK8_46 / 15MAK8_7 and 15MAK8_74 / 15MAK8_7 were least similar with a distance of 0.56 between them.

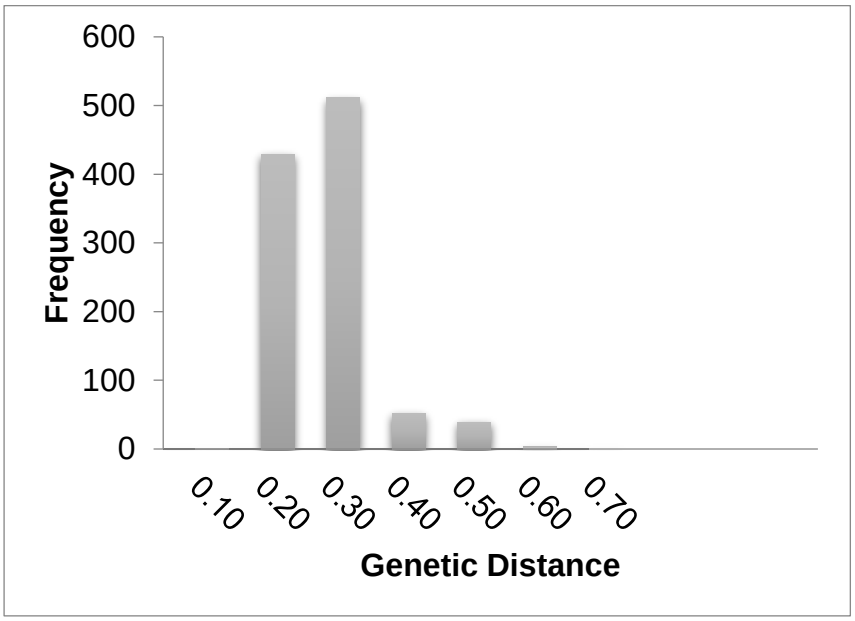

Figure 4.8: Genetic distance distribution of the 46 maize inbred families.

4.3.3 Cluster analysis based on molecular markers

The dendogram of 46 maize inbred families is shown in Figure 4.9. The SNP markers were effective in categorizing the lines into different clusters. The families were grouped into three major clusters I, II and III, whereby cluster I was the largest cluster (26 families). Cluster I and II were further divided into two sub-clusters. Cluster III had only four families and cluster II had sixteen families. The clusters were distinct by 98% using cophenetic correlation (r).

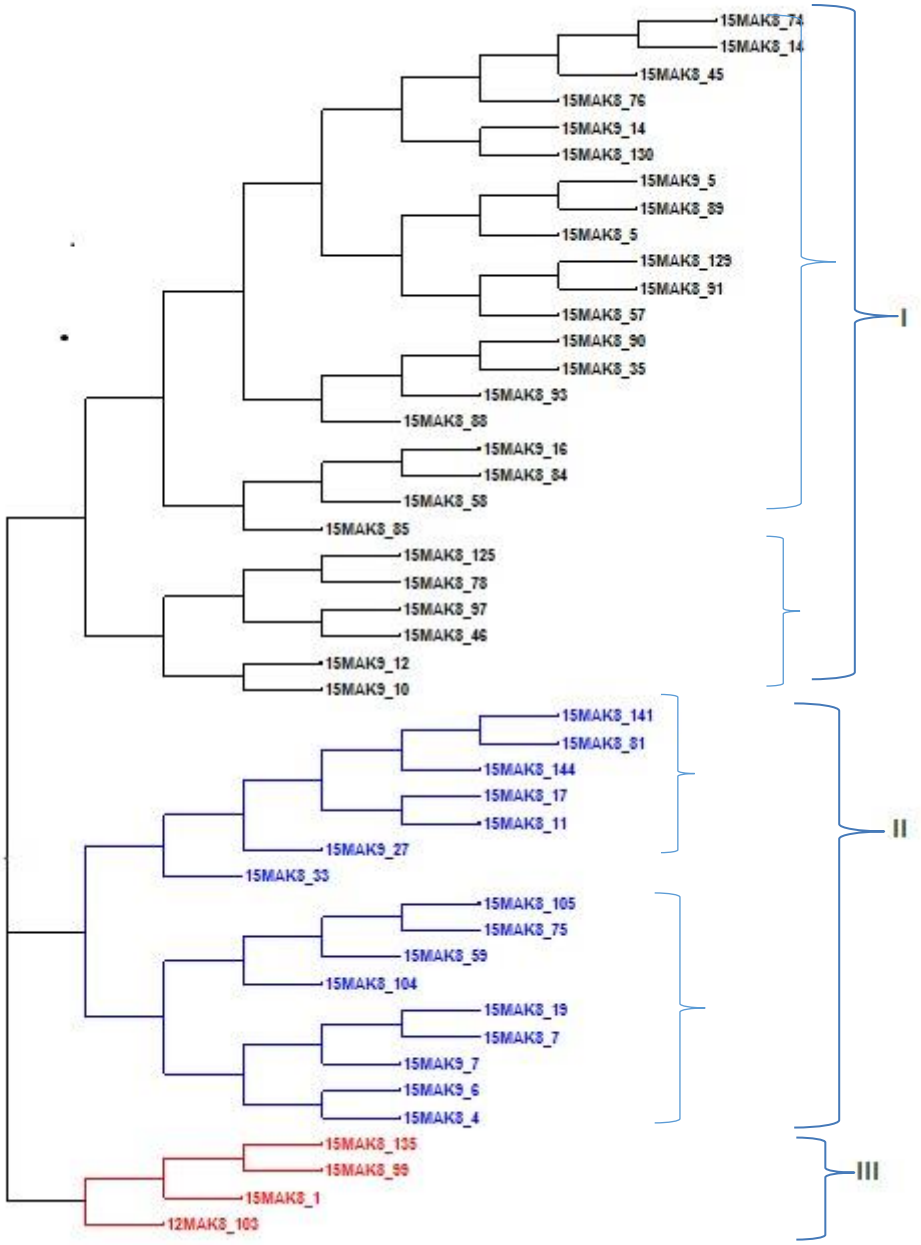

Figure 4.9: Dendogram of 46 maize inbred families based on 391 SNP markers.

CHAPTER FIVE

DISCUSSION

5.1 Introduction

This section provides interpretations for the results and further explains the data presented under the results section

5.2 Phenotyping

5.2.1 Site performance

The results of the analysis for the two sites revealed significant variation among the families for all traits (Table 4.1 and Table 4.2), but they were dependent on the environment. This support the study that was done by Mitrovia et al. (2012) who reported that the highest percentage of variation in their study was explained by environment. The presence of genotypic variation is good for breeding (Hallauer and Miranda, 1988), since it provides the basis for selection (Anshuman vashistha et al., 2013).This indicates that selection for highly productive and early maturing traits could be effective in this population of 50 inbred families. However the inbred family X site interaction mean squares were significant ($P< 0.05$) for grain yield, ear prolificacy, root lodging, stem lodging, grain moisture and yield score, and was highly significant ($P<0.001$) for the anthesis-silking interval, indicating that performance of the families would depend on the site of selection. This might complicate the selection but it also provided an opportunity to select families that performed at both sites. These are stable families that can provide inbred progenies with broad adaptation.

The coefficients of variation (CV) of the 13 traits that were studied explained that significant variation existed in almost all traits at both sites. The CVs were high (>20%) for anthesis-silking interval, root lodging, stem lodging and ear rot at Ukulinga and grain yield, ear prolificacy, anthesis silking interval, ear height, root lodging, tem lodging and ear rot at Cedara. The other traits at both sites had low CV (<20%), this was expected because the families had undergone inbreeding depression resulting in fixation of recessive genes and

that increases homozygosity within the lines. This was previously reported by Ogunniyan and Olakojo (2014). Since the families were developed for similar characteristics, the minimal differences among the lines based on the traits with low CV were expected.

The difference in the mean value for each traits revealed distinctiveness of each family since they were derived from a segregating F_2 population. The standard errors were low for the traits at both sites, which indicated that the lines shared almost the same pattern of gene actions. This was reported in the study that Maphumulo et al. (2015) conducted. The LSD (0.05) showed that the line were different and with that there is still a chance to perform selection of genotypes for advancement. This is in accordance to the study that was conducted by Ogunniyan and Olakojo (2014).

5.2.2 Mean performance

Most of the high yielding families were the late maturing ones whereas those that matured early had low yield. This supports the statement that was made by Gasura et al. (2010) that despite the ability of early maize to provide food early, it is associated with low yield potential that was estimated at about 20% to 30% less than the late maturing varieties. However, this is more profound under conditions of favorable rainfall and temperature. Comparing the average mean for grain yield from the two sites: 2.10 t/ha (Ukulinga) and 0.70 t/ha (Cedara), the above statement is supported. The conditions at Cedara for season 2015/2016 were unfavorable for the growth of maize which then contributed to yield being very low even for late maturing lines. The plant leaves were damaged by hail storm at Cedara and that compromised grain yield as it is known that upper leaves contribute assimilates to grain yield. Also day temperatures were low at Cedara that compromised the yield as well. The family 15MAK8-74 was an exception at both sites. It was early maturing and high yielding. It appeared in the top ten of best yielding lines and of early maturing lines at both sites. It exhibited progressive stability in different environments which is desirable. This is the family that will contribute to ideal lines that will be easy to produce seed and maintain it in a breeding programme. The inverse proportionality between grain yield and early maturity complicate and impose so much challenge in breeding for high yield in early maize. The knowledge of genetic variability in grain fill rate and grain fill duration would help (Gasura et

al., 2010), but these two traits need complicated and laborious destructive sampling methods so as to establish the lag, linear and final phases of grain filling (Lee and Tollenaar, 2007).

5.2.3 Heritability and Genetic advance

The data from both sites and for combined locations exhibited moderate heritability for grain yield. The results of the study contradict with Maphumulo et al. (2015) who previously reported high heritability for grain yield. This is because the families that were used in the study were not yet fixed, they are still undergoing segregation and also the environments were different. This implies that genetic variation was lower than environmental variation for grain yield at both sites meaning that environmental variation contributed more than genetic variation to the performance of the test inbred families. However moderate heritability (38%) for grain yield have also been reported by Asghar and Mehdi (2010). Heritability alone provides no indication of the amount of genetic improvement that would result from selection of individual genotype, therefore the knowledge about genetic advance in line with heritability is most useful (Ogunniyan and Olakojo, 2014). For grain yield at both sites, the genetic advance was low. This is in accordance to the previous study by Maphumulo et al. (2015) where the grain yield genetic advance was low. This indicates that yield performance still needs to be improved.

Estimates of heritability for secondary traits varied between sites indicating the role of GXE effects. At Ukulinga it ranged from as low as 6.50% to 69.75%, where ear rot had the lowest heritability and anthesis date had the highest. At Cedara the lowest was for stem lodging (2.86%) and the highest was for anthesis silking interval (66.23%). With the results that were obtained for secondary traits heritability there is still a need to identify the traits that needs to be aimed at for the improvement of the families. High heritability value for ASI at Cedara is in contrast with the report that Bello et al. (2012) presented and high heritability of anthesis date at Ukulinga is in line with Bisawas et al. (2014) who reported a high heritability for anthesis date. Therefore anthesis silking interval at Cedara and anthesis date at Ukulinga being the traits with the highest heritability outlines that they are suitable target traits for the improvement of yield. Their genetic advances were low though, which means a more reliable conclusion about them cannot be made as yet. The other traits like ear prolificacy, silking date, ear position, ear height, plant height and grain moisture had moderate heritability at

both sites except for grain moisture content at Ukulinga, where it had a low heritability (25.09%). Low to moderate heritability of grain moisture content revealed that genetic variation was small and it could not respond to selection (Maphumulo et al., 2015). Traits with relatively moderate heritability like ear prolificacy (56.53%), silking date (57.94%), ear height (45.79%), plant height (51.88%) in Table 4.11 and anthesis date (42.65%), silking date (49.85%) in Table 4.12 pin point that variations were transmissible and there is potential for developing high yielding varieties through selection of desirable plants in succeeding generations (Mhoswa et al., 2016). These traits had low genetic advance at both sites, this is consistent with previous studies by Anshuman vashistha et al. (2013) whereby moderate estimates of heritability along with low genetic advance were observed. Therefore improvement of these traits through selection is limited. In addition, there are traits that really need improvement in their performance and with that grain yield will be improved also.

5.2.4 Coefficient of variation

The study of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) is useful for comparing the relative amount of phenotypic and genotypic variations among different traits and also estimate the scope for improvement by selection. The reliability of a trait to be selected for breeding programme among other factors is dependent on the magnitude of its coefficient of variation especially the GCV (Bello et al., 2012). However, the difference between genotypic and phenotypic coefficient of variability indicate the environmental influence. The results in Table 4.11: and Table 4.12 depicted that PCV values were higher than GCV for all the traits, suggesting the significant genotype X environment interactions in the expression of the traits. Similar results have been reported by Ogunniyan and Olakojo (2014) and Maphumulo et al. (2015). A high GCV to the PCV is desirable in breeding work but it is difficult to obtain such ratio especially for quantitative traits since GXE has a huge impact on them.

5.2.5 The frequency distribution of families for yield and secondary traits

The results showed that the distribution of grain yield and secondary traits differed from both environments due to that GXE interaction effect was significant, meaning that the two environments had different conditions. Many inbred families at Ukulinga had high yield when

compared to those at Cedara, which only yielded as high as 1.8t/ha. This could be because Ukulinga had favorable conditions during 2015/2016 season making it a favorable environment in contrast to Cedara which had unfavorable conditions for 2015/2016 season due to the storm that took place during plant growth as well as less heat units due to lower day temperature. This affected flowering badly which is a very sensitive stage of growth. The storm damaged the leaves and reduced the photosynthetic leaf area and its duration. There was also drought at Cedara during the vegetative stage of growth, therefore the plants did not get as much nutrients as they needed while still young. Many inbred families produced pollen early at Ukulinga (69-71 days), whereas at Cedara they produced it later (75-77 days). This was expected though since Cedara is generally cooler than Ukulinga due its high altitude hence plant growth cycle would be longer. Grain moisture content data was continuously distributed at Ukulinga deducing that the trait was governed by minor genes with quantitative effects which was previously reported by other researchers (Maphumulo et al., 2015). The normal distribution of grain moisture content data explains the little effects of GXE at Ukulinga whereas at Cedara the data were distributed to the right (Figure 4.4), having high grain moisture content. This once again pin point how unfavorable Cedara was during 2015/2016 summer season. Very few inbred families at Ukulinga were prolific while at Cedara none were prolific indicating that the environment played a crucial role in governing the traits.

5.2.6 The relationship between phenotypic traits

Ear prolificacy and ear height were highly significant ($P<0.001$ and $P<0.01$) and strong, positive correlation with grain yield at both sites. This indicates that the favorable genes controlling these traits could be utilized for improvement of the test lines in future breeding programmes and that increasing the expression of these traits can positively influence grain yield potential. These results are in harmony with those that were obtained by (Maphumulo et al., 2015). They reported that ear prolificacy was highly significant and positively correlated with grain yield but these findings are in contrast with Sreckov et al. (2011) report. A strong correlation between grain yield and ear prolificacy was reported by Munyiri et al. (2010) to be the most important yield component of maize, especially under stressful conditions, it contributes more to yield. This was supported by the study that Ribaut et al. (2009) did where they reported that under drought, grain yield was strongly positively

associated with the number of ears per plant. In the review, a strong relationship between ear prolificacy and grain yield was also mentioned.

Grain yield showed negative correlation with yield score and anthesis silking interval in both sites, however at Cedara the yield score was highly significant ($P<0.001$) and at Ukulinga it was significant ($P<0.05$). This is desired since it is an indication that the ears of the test families were good visually. Anthesis silking interval was significant ($P<0.05$) at Cedara and significant ($P<0.01$) at Ukulinga. This is because of the different conditions in the two sites. These results are in line with Juma et al. (2013) who reported anthesis silking interval to have a negative correlation with grain yield. In the literature a strong correlation between yield and anthesis silking interval was mentioned but the study showed the correlation between the two traits to be weak. With negative correlation on anthesis silking interval is favored to obtain early maturing genotypes and targeting this trait would be effective for indirect selection for grain yield.

There was non-significant but negative correlation between grain yield and root lodging at Cedara. This is because if the plant is root lodged, its ability to extract nutrients from the soil is reduced. An environment that has heavy rains matching with wind is highly likely to expose plants to root lodging. The storm that took place at Cedara caused the plants to lodge at their root. At Ukulinga there was weak, negative correlation of grain yield with stem lodging, ear rot and silking date. This is in contrast with Selvaraj and Nagarajan (2011) who reported silking date to be positively associated with grain yield.

Ear prolificacy had a negative correlation with anthesis-silking interval. As stated in the literature by Ribaut et al. (2009), these two traits are believed to have a strong correlation with grain yield but with the outcomes of the study, these two traits could not be used together as an indicator for high yield as they affect the performance of each other negatively.

5.2.7 Path coefficient analysis

Yield score on both sites was the most important trait of grain yield as it had the highest level of significance with a negative effect. This was desired because a lower score indicated that ears were well filled with good appeal. The presence of high significance and positive association between grain moisture content with gran yield at Cedara shows that the results of regression are in line with correlation results. This indicated that high grain yield potential was associated with high grain moisture content because most of the families were skewed towards late maturity in this population of F_4 families. All traits with significant regression can be included in the selection criteria.

Path coefficient analysis was conducted to determine the nature of relationship between grain yield and its contributing components and then identify those components with significant effects on yield for potential use as selection criteria. Path analysis shows direct and indirect effects of secondary traits on grain yield (Farshadfar, 2004).

Grain moisture content had high significant, positive direct effect on grain yield at Ukulinga. Thus grain moisture content could be used in the selection criteria since it is an indicator of early or late maturity. Selecting for high grain moisture content would result in plants that are late maturing since it takes long for the grain to dry on the cob. Ear prolificacy had a positive and direct effects on grain yield even though it did not impose strong effects, indicating that yield of families would increase by selecting for many ears per plant. This contradicts previous report by Rahmani et al. (2014) who found that ear prolificacy had a negative direct effect on grain yield. Ear height can be used as a primary selection criterion when breeding for grain yield for the target environments that are represented by the Ukulinga Farm since it had a moderate, positive direct effect on grain yield, indicating that yield could be improved by selecting for high ear placement on plants.

The Cedara site revealed the highest direct, positive effects of ear height on grain yield which is consistent with Pavan et al. (2011) who reported high and positive direct effects of ear height on grain yield. Ear prolificacy had the highest direct and negative effects on grain yield potential. The same results were obtained by Rahmani et al. (2014). Path analysis

indicated that ear height and yield score were the major contributor to grain yield potential through their direct effects on grain yield, therefore they should be given high weightage in a selection process aiming at improving grain yield.

For the convenience of interpretation of data the indirect effects were ranked using Lenka and Misra (1973) method, where 0.00-0.09 = negligible; 0.10-0.19 = low; 0.2-0.29 = moderate and >0.3 = high path coefficients. In this regard at Ukulinga, all traits exhibited negligible indirect effects on yield potential, which means indirect selection for these traits would not improve the grain yield of the families. The number of plants contributed more to grain yield followed by ear prolificacy when looking at the total correlation to grain yield, indicating that a high plant population would be required to realize high yield potential from the these families. This support the correlation analyses where ear prolificacy was strongly correlated with grain yield. These two traits had more significant role on grain yield, so they must be considered in the improvement of yield. At Cedara, the traits ranged from negligible to low indirect effects on grain yield potential.

5.3 Genotyping

5.3.1 Marker Characterization and Genetic diversity among families

Having knowledge about genetic diversity in a breeding programme is crucial for early diagnosis of genetic narrowing of heterotic pools and designing of competent strategies for broadening them. This will help assure future gains in yield performance (Mikel and Dudley, 2006). Of the 50 lines evaluated in this study, 46 were amplified by 391 SNP markers. The other four were consistently producing spurious data, therefore they were removed from the analysis. Molecular basis of polymorphism and their distribution across the genome differs from one marker to the next (Singh et al., 2013). The utility of SNP markers in crop improvement depends on the quality of information they provide with respect to parameters for genetic diversity and population structure.

The total number of polymorphic alleles amplified per locus over all genotypes ranged from 32.0 to 53.0, while the mean number of alleles per locus for the whole set of genotypes was

39.0. The values in the study outline the wide range of genetic diversity represented by the genotypes since the two lines that were crossed are from different geographic origin. They were quite divergent – a temperate line crossed with a tropical line yielded high level of diversity from which selection would be effective. It is also important to remember that the total number of alleles reported in diversity studies is usually proportional to sample size. The effective number of alleles per locus (N_e) was lower for all the chromosomes when compared to the total number of alleles per locus (N_a). This observed difference between the total number of alleles per locus and number of effective alleles per locus was due to variation in the frequency of major alleles among genotypes. At a single locus, the distribution of allele differs among genotypes.

Average gene diversity and polymorphic information content (PIC) values revealed by SNP markers in this study were 0.49 and 0.48 respectively (Table 4.16). Compared to the previous studies on maize the average gene diversity of the present study was low. In the work that Legesse et al. (2007) reported, average gene diversity was 0.59, indicating the high levels of polymorphisms in the inbred families. When PIC values are moderate, it is an indication that markers can uniquely and detect the polymorphism rate at a specific rate (Smith et al., 2000; Legesse et al., 2007). Senior et al. (1998) and Xia et al. (2004) reported PIC of 0.59 using 70 SSR markers and 0.60 using 79 SSRs, respectively, which are much higher than the one that was obtained in the current study. However Legesse et al. (2007) found a lower PIC value (0.33).Therefore the genetic diversity data in the study can be considered reliable.

5.3.2 Genetic distance

There was genetic variation between the maize inbred families. The family 15MAK8_90 / 15MAK8_35 were the most genetically close, with 15% distance between them. These two inbred families were clustered together, meaning they are very closely related. They have less potential of producing best performing hybrids when crossed. This is so, because crosses between closely related inbred families result in inbreeding depression, which is the decrease in the expression of quantitative traits (Maldonado and Miranda Filho, 2002). Conversely, the largest distance was found between 15MAK9_5 / 15MAK8_7, 15MAK8_46 / 15MAK8_7 and 15MAK8_74 / 15MAK8_7. This explains why they were clustered in different

clusters. These inbred families have different gene frequency. Therefore they have the ability to produce best performing hybrids.

5.3.3 Cluster analysis based on molecular markers

The inbred families (46) were clustered into three different genetic groups, using the 391 SNP makers, indicating that there was diversity available for selection. The SNP markers showed their effectiveness since they could discriminate the inbred families according to genetic backgrounds relative to the parents used in the cross of origin (tropical and temperate). The inbred families were assigned into three different clusters, representing inclination towards the tropical maize parent, temperate maize parent and the intermediate those with equal genes of both parents. The early families were spread across the three clusters. Three inbred families in cluster III represented early lines and were therefore inclined towards the temperate parent that was early. These families have the earliness genes that would be fixed in the programme. In cluster II few were early but most of them were intermediate maturity, still having genes from both parents at an almost equal state, and consist of recombinants that would show phenotypes of both the temperate and tropical parents. The Cluster I comprised the inbred families that were both early and late maturing, therefore cluster I has families that are either close to the tropical parent or temperate parent.

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CHAPTER SIX

GENERAL OVERVIEW OF THE STUDY

6.1 Introduction

This chapter makes an overview of the completed study by summarizing the major objectives and highlighting the major findings. The implications of these findings and recommendations are discussed.

6.2 Findings from the literature

A survey of the literature has revealed that,

i. There is very limited research that has been done regarding the level of yield in tropical and temperate early maturing maize.
ii. Genetic diversity of early maturing maize has not been given much attention.
iii. Most of the diversity in maize is poorly understood and underutilized in modern crop improvement programmes.
iv. Early maturing maize varieties are ideal for intercropping since they compete less for moisture, light and nutrients than late maturing ones.
v. There is limited information to establish drought adaptive traits of early maturing maize germplasm.

6.3 Findings from the research

The study was successful in phenotyping and genotyping F_4 families. The information would be useful to improve the breeding programme.

i. The study revealed significance of mean squares for all traits on both sites. Indicating the presence of genetic variability among the F_4 families for all the traits with the influence of environmental effects.
ii. The F_4 families: 15MAK8-74, 15MAK8-75 and 15MAK8-57 were among the top ten high yielding families on both sites but only 15MAK8-74 was among the top ten of early maturing lines when using phenotypic data. This confirms that it would not be

easy to find families that combine both high yield and early maturity in the segregating population.

iii. The data from both sites and for combined locations exhibited moderate heritability for grain yield. This is because the families that were used in the study were not fixed, they were still undergoing segregation and also the environments were different. Within family variation was large given that the materials were still at the F4 stage with only three cycles of inbreeding.

iv. Ear prolificacy and ear height were highly significant ($p<0.001$) and they exhibited a strong and positive correlation with grain yield at both sites. This indicates that the favourable genes controlling these traits could be utilized for improvement of the population in future breeding programmes and that increasing the expression of these traits can positively influence grain yield.

v. Consistent with the literature there was a significant relationship between grain yield and most of the secondary traits, with implication for breeding strategy. These traits would be exploited to improve yield in the temperate x tropical maize population.

vi. Ear height and yield score were the major contributors to grain yield through direct effects on grain yield qualifying them to be selected as a significant traits that would be targeted during selection to boost grain yield for the target environments.

vii. SNP genotyping produced reliable genetic data that revealed significant variation between the F_4 maize families, indicating that selection of families to advance to the next generation would be effective. Three clusters were observed hence selection could be done within clusters to maintain some level of diversity between the target lines to be extracted from this population.

viii. Using genotypic data, the early maturing families were located across the three clusters with cluster III having three out of four families that were early maturing and clustered together, indicating that the markers could be used for background selection.

6.4 Conclusion

From the completed research conclusions could be drawn with respect to the four objectives of the study.

The first objective of the study was to phenotypically characterize F_4 families derived from temperate x tropical maize F_2 cross. The results from the study revealed that 23 families out

of 50 families were early maturing, that was concluded by looking at their average rank. The 23 that were early maturing, 14 of them are associated with high yield.
Secondly, the study aimed to genotype F_4 families derived from temperate x tropical maize F_2 cross in order to reveal genotypic variation within the population. The early maturing families were spread across the three clusters. Cluster III had three families out of four that were early. Most of the other early families were in cluster II. The majority of the families had a distance of 0.30. This indicates the presence of genetic diversity among the inbred families.

Thirdly, the study was designed to select F_4 families for earliness using phenotypic data and genotypic data. The phenotypic results from the concluded study showed that 14 families were early and high yielding. Genotypic data identified three early maturing families (15MAK8_135, 15MAK8_99 and 15MAK8_1) that were clustered together indicating that they were inclined towards their temperate parent. Only 15MAK8_99 among the three was high yielding which indicate that it is also carries the genes of the tropical parent.

Fourthly, the study aimed to determine the relationship between grain yield and secondary traits in the maize inbred families derived from a temperate x tropical F_2 population. The study results showed that ear prolificacy and ear height can be used in the improvement of the test families since they were positive and highly significantly associated with grain yield. Yield score is another trait that was desirable in the study as it had a negative correlation and direct effect on grain yield. This was an indication that the ears of the families were good and could be used for planting in advanced evaluation of the families. The direct effects had the influence on grain yield when compared to the indirect effects, therefore only direct effects can be used for further improvement of yield.

Therefore using both methods was effective since each method highlight effectiveness of the other. With phenotypic studies general performers as well as specific performers were obtained. While with genotypic studies, the efficiency and precision of conventional plant breeding were highlighted.

6.5 Recommendations

The following recommendations could be made.

i. The early and high yielding lines like 15MAK8-74 must be evaluated further for disease resistance to confirm their complete performance before recommendation to farmers. This could not be done in the current study because time was very short.

ii. From the results some of the lines that were early and high yielding showed genetic diversity, hence these lines can be used in the production of early maturing, high yielding maize hybrids.

iii. Observation of GXE interaction as reflected by significant family x sites interaction effects in the completed study implies that performance of the inbred families was dependent on the sites. Therefore there is need to evaluate all the inbred families in many locations and years so as to determine their stability across different tropical regions. This could not be possible for the current study due to limited funds and resources.

iv. A heterotic tester should be found to determine the GCA and SCA of the potential superior lines that can be extracted from each of the three genetic clusters that were observed.

v. It is important to understand the nature of gene action operating for grain yield as this will help in developing effective breeding strategies therefore gene action for superior families has to be studied.

APPENDIX

Table 3.3: SNP markers used to genotype the F_4 inbred families.

SNP ID	SNP No.	Allele Y	Allele X	Sequence
ae1_7	9030686	G	A	TAATAGGCCATATTC[A/G]TTCTCGGTTTATAC
csu1138_3	9030563	G	A	CGGAGGGTGGTAGAT[A/G]GGAGTCGGTGATCC
glb1_2	90071	T	A	TGTTGTTKYSTCGTG[A/T]CTAGGCGAGGGAGT
lac1_3	9030074	G	A	GGTCAATCCAGCATT[A/G]TATGTAACACCAAC
PHM11000_21	9030212	G	A	TTCGAAGGAGTCAAG[A/G]TATAAATAACAGGG
PHM1190_3	9460168	G	A	CAAGCATGGTCTCCA[A/G]GTATCCTTGTGCTT
PHM12323_17	9460007	G	C	TCAGTTCTGGTTCCT[C/G]CTTCGCGTAGGTGA
PHM12706_14	9030523	G	A	CTCCTCGACCAAGAA[A/G]GCACGAATGATTCT
PHM12749_13	90296	G	C	CAAGTGGGGAATTGT[C/G]AAAGCTGCTGAAAG
PHM12794_47	9460164	T	A	ACAGTGATGCTGAAA[A/T]GCTTCACCGTGATT
PHM13360_13	9460081	G	A	CCAGGCATCCATCCA[A/G]CCAACGCTTTGCTA
PHM13440_13	9460071	G	A	CCAGATGATATACTC[A/G]ATAAGATCGCTCAA
PHM13942_7	9030085	G	A	CCAGGATGAGTTTGC[A/G]ATCTCGGCGCGGTT
PHM1438_34	9030260	G	A	CCAGTCAACAGGCAC[A/G]CCATGGATTTGGTT
PHM15251_3	9030384	G	A	CTCATCAGGAGCATC[A/G]TACTCATCATCTTC
PHM15331_16	8170033	G	A	CGTCTTCAGGCGCTC[A/G]CTGCGGTTGGACGT
PHM15501_9	9030091	G	A	GATAAGGCATACATT[A/G]CAAATTCAGATCAA

SNP ID	SNP No.	Allele Y	Allele X	Sequence
PHM15964_16	9030751	G	A	CCTTGAATGTGGGTC[A/G]CGTCTTATACACAG
PHM16788_6	9030115	C	A	TCTTCTTGGTAATAT[A/C]TTGATCCCTTCTCT
PHM16854_3	9460149	G	C	GTTCTTGACAGGATG[C/G]CGGTGAGCGATCAC
PHM17210_5	9460086	C	A	CATCAAACCATCAGA[A/C]TAACAGGCTTTCTG
PHM1725_34	9460039	C	A	GACATCTTCGAACAG[A/C]AGCCCAAGTAATGC
PHM174_13	9460008	G	A	AAGGATGTAGGCGTC[A/G]TCTGGAGCATCAAA
PHM1752_36	9030217	G	A	ACGTGTGTACTGATC[A/G]TGGTTAAGCACGTA
PHM1834_47	9030544	G	C	CGTCATCGTACTCCT[C/G]CTCGTCCTCTTGCG
PHM18513_156	9030647	G	A	CCACCTACGTTCAAA[A/G]TGAATTGGCCTATC
PHM1870_20	9460135	G	A	GCGTTACTTGCTGTA[A/G]TAGAGGGGCAACAG
PHM1899_157	9460153	G	A	GCCCTGACTGGCGGT[A/G]TATGTGATCTGCAT
PHM1911_173	9030437	G	A	AGCTCTGGTTGCACG[A/G]TCGAGTTAACCGGT
PHM1932_51	9030196	G	A	TGCCTTGTGGAAGTG[A/G]TCTAACAAGTGCTT
PHM1968_22	9460010	G	A	ATGATCGCTTCTTCA[A/G]CTGCTGCCTCCTCC
PHM1971_20	9030121	G	C	AGAACTAGCCAGTTA[C/G]CTCAAATCATCAGT
PHM229_15	9030077	G	A	CTGGCAGGAGCAATC[A/G]CCCTAGGTGAATCA
PHM2343_25	9030716	G	A	AAATAGGTAATGCAG[A/G]CACACCTCTGGGGA
PHM2350_17	9030477	C	A	CAATATGTTTGTGTT[A/C]TGCAGACCTTCAAT
PHM2423_33	9460102	C	A	AGATAATAACAAAAA[A/C]AAATCACACAACAA

SNP ID	SNP No.	Allele Y	Allele X	Sequence
PHM2658_129	9030124	G	A	GGTACCATCTGATGC[A/G]TTTGAGAATGATTA
PHM2691_31	9030349	G	A	GTTTGCAGCAGGCGG[A/G]CCAGCAGCTGTCTG
PHM2749_10	9030451	G	A	CAAGAGGATGAGAGC[A/G]CACTATGAAGTCCT
PHM2770_19	9030478	C	A	AGCTGCTTTAGAAAT[A/C]TCTCCCTCCAATTC
PHM2828_83	9620022	T	C	ATTATGTTATCGTCA[C/T]TGGCTGTACCTTTG
PHM3055_9	16260019	G	C	AARTTACTGTCCATA[C/G]CTTTTYTCTGTCCC
PHM3147_18	9460013	G	A	GGCTTGGTTCCTAAA[A/G]AACAAAACAAAGGT
PHM3155_14	9460122	G	A	AACAGGATGTGATAC[A/G]ATGTAACGTTGATT
PHM3309_8	9030070	G	A	CCAAGGGCCTGAAGG[A/G]TACAGAGGGATTTA
PHM3342_31	9460094	G	A	TGCTCCAGCCAAACC[A/G]ACGTGGAAGTGTTA
PHM3435_6	9460172	G	A	CTGCGATAGTATCCA[A/G]GCTCAAGGAGTTCC
PHM3457_6	9030149	T	A	GGATGTCCCAAAGCT[A/T]GACACGATCCTCAC
PHM3466_69	9030313	G	A	ATCTTCTGAAACAGC[A/G]GCTCAACCCAGGGT
PHM3512_186	9460146	G	A	TTGGGAGGGATCACA[A/G]CGCATGCATAATTA
PHM3587_6	9460115	G	A	TTAAACCCTGCACAT[A/G]ACGTGAATATGTAT
PHM3626_3	9460065	G	A	GCCATTGACACCAGA[A/G]ATGTGATGCAGATG
PHM3668_12	9460061	T	A	AAAGCATGAATCATA[A/T]AGTTATGTTGTTTT
PHM3736_11	9030294	G	A	GAAGAGCAGTGAGAA[A/G]CTGGTGAGGAGATT
PHM3762_18	9030376	G	A	GAAGAAGGTTAGGAG[A/G]CAATTTCCAGTAGT

SNP ID	SNP No.	Allele Y	Allele X	Sequence
PHM3844_14	9030308	T	A	TAAAGCACTCACCAG[A/T]CGCGCTGTGTGGAG
PHM3856_10	9030634	G	A	TTGCCCCTTGCGATT[A/G]ATTGTGTGCCGCTT
PHM3922_32	9030673	G	A	GACACACACGAACAT[A/G]TACACACATACAAG
PHM3963_33	9460120	G	C	TAAAACCAGCATCGT[C/G]GCCACCGAGTCCGC
PHM4066_11	9030081	G	A	TGGACCACATCCATC[A/G]CTTTCTTCTTCGCA
PHM4080_15	9030468	G	A	GATGGTGCATGGAAC[A/G]CCTAGGACATAGGA
PHM4117_14	9030734	C	A	TAGAAGATAAGCTAT[A/C]AGAAATCAAGAACG
PHM4125_11	9030754	G	C	TGCGACGAAGGAAGG[C/G]GCCGAGGAGCCTGG
PHM4134_8	9030463	G	C	GACGGAGAGGCTAGT[C/G]GTTATGAGGGGCGA
PHM4145_18	8170059	G	A	CTGTCTGTCCAAGTA[A/G]CAGCAGGGTTTAAG
PHM4165_14	9460157	T	A	CTGTAGATTCATAAT[A/T]GCAGGTGACAAGGG
PHM4259_5	9460087	G	A	TCCGGTTCCTCTCCC[A/G]GAGCTGAACCTGCA
PHM4353_31	9030577	C	A	AACCTTCGGCGTAGT[A/C]TGATGTACAGTGAA
PHM4468_13	9460161	G	A	TGATGATGAGAAGAA[A/G]TCTGAGACTGCTAT
PHM4503_25	9030324	C	A	CTGATATTGTGGTGA[A/C]GTGGAGTACCATGT
PHM4597_14	9030276	G	A	GCTAATATCTGGCGC[A/G]TGCGAGCAGACCGG
PHM4786_9	9030300	G	A	ATGGCGAGGAGCAAT[A/G]TGTATAGCTTCCCC
PHM4818_15	9030247	G	C	GCTCCATTGCTCCTT[C/G]AGGATATCAAGGCA
PHM4880_179	9460064	G	A	GTACAAACCAGTCTC[A/G]AAGCAGTAAATTCG

SNP ID	SNP No.	Allele Y	Allele X	Sequence
PHM4955_12	9030534	G	A	TTAACCAAGTGTCAA[A/G]CTACTGCATCTGCC
PHM5181_10	90429	T	C	CTCAACCAGCACAGG[C/T]ACAGGCTAGTTCAT
PHM5435_25	9030429	G	A	TGATGGCCGAGAGGG[A/G]AATGGCTACCAAGT
PHM5502_31	9460098	G	A	ATGTACACAATCCGG[A/G]AGACTTTCACCCTA
PHM5572_19	9030500	G	A	CAATGAAATGGAACA[A/G]GAAACATCGTTTTC
PHM5622_21	9460026	T	A	TCTGGAGGAAGAAAC[A/T]GAAGGCGATGGCAA
PHM5740_9	9030259	G	A	AACGTTCAACTCAGA[A/G]CACACCTTGGATAT
PHM595_30	9460037	C	A	TTGTGTCCTACTTGC[A/C]AAGGTAGATAAATT
PHM597_18	5960260	G	A	GTTCTTGTAGTTCGC[A/G]GAGTCGTCCATGGC
PHM6111_5	9460060	G	A	ACACAAAGTTGCAGT[A/G]TGCCACACAACCAA
PHM662_27	9460133	C	A	CCAGTTCCTCGACAC[A/C]GCCTACATCAGCGG
PHM697_21	9030610	C	A	CATGATCTTCTTCCT[A/C]TCGTCCCGTTTTGG
PHM759_24	9460027	G	A	CCCAGGAAAACTAGA[A/G]CTTTTCTGTGCATG
PHM765_24	9030303	G	A	ATGGGCACGGATAGA[A/G]AATATGACCATCCT
PHM7916_4	9030284	G	A	CTCTTCACTGTCAGC[A/G]TCACCATCGACCTC
PHM793_25	9460056	G	C	AATAGCTATTGTTAC[C/G]CACTGGTTCTTCCG
PHM816_29	9030467	G	A	AGCTTGCCCACACCA[A/G]CCATGGCCATCGTC
PHM934_19	9030494	G	A	CAAACTTTTCCGGGT[A/G]GAGGTATATTTCCT
PHM9374_5	90335	T	C	GCATTTACATGCTAA[C/T]GGGTTCAGTTTATT

SNP ID	SNP No.	Allele Y	Allele X	Sequence
PHM9914_11	9620034	G	A	GGAGATGCTATCTGG[A/G]TTACACAAGACTGT
PZA00058_1	90300	G	A	CCTCAAGGTAGCTTT[A/G]TTTTCGTTAAGGAT
PZA00084_2	90270	T	C	GAACAAATTCGTGCA[C/T]CAGTGAATATAAGG
PZA00109_4	9460089	T	A	ACAGTTGCACTTTGA[A/T]TCGACCTTTGGAGA
PZA00148_3	9460147	C	A	GAGGTGCTGCATGTA[A/C]ACCGCCTCCGGCAC
PZA00175_2	90035	T	A	TACGGCATCGGCAAC[A/T]TCCTCAGCAGCTTC
PZA00186_4	9460093	G	A	ACATCAGCAGCTTTG[A/G]GCCKGGCTTTATTC
PZA00214_1	90252	T	A	GTCAGCACTGAGTCG[A/T]TTGAGGTGCTCGTG
PZA00218_1	90531	G	A	AATATTGGCGGTAAC[A/G]CAAGCTRATGTCGT
PZA00219_7	9460092	G	A	ATCTAACAAGCCCTG[A/G]CCAGAGAGCAAAAG
PZA00256_27	16260105	T	C	TCCCAACATGCTCAG[C/T]GAGGGTGGCCACCA
PZA00282_19	9030550	G	C	TGTTCTATCATCAGA[C/G]RCGAGGCCCTTGTC
PZA00300_14	9030336	G	A	TATCAATGTTTCATT[A/G]TTGTTTTGTTCAAT
PZA00323_3	90340	T	C	CCATGAAGGCTTTTT[C/T]CCTCGGGAGTCTCA
PZA00337_4	9030554	G	C	TCTCGAAGCGTATTT[C/G]CATTCAGTGTCAGT
PZA00352_23	9030140	G	A	ATGACACTTTGTTTG[A/G]CAGCTACATGAAGA
PZA00355_2	9460169	G	C	CGAACCAAATGTGAT[C/G]ACAGCAGCTAAYCA
PZA00379_2	8170004	T	C	CAGAGCCTACCTGGA[C/T]GGTGCCCAGGTTGG
PZA00390_7	9030472	G	A	CAGCATCTGTTACTT[A/G]TGCTAAGCGCTACA

SNP ID	SNP No.	Allele Y	Allele X	Sequence
PZA00405_6	9030089	G	C	CGACCAAATTGAATT[C/G]GTCATTTACGCCAG
PZA00413_20	9030210	C	A	AAGCAAACCATCCTC[A/C]GCACCGGATTCTGC
PZA00418_2	9030488	G	A	GGCCTGAGTAGAAGA[A/G]CTTGATCGTCAGCT
PZA00424_1	9030240	G	A	CGCCAGGGAAGCAAA[A/G]TTCTCCGCTCCATT
PZA00436_7	5960166	T	C	TCATAAGCTGACAGA[C/T]TACCTCAGTTACCA
PZA00440_1	9460165	G	C	GCGCGGGTCGTTGGA[C/G]GGACGATGATAGAG
PZA00455_14	9030565	G	A	TACTCTCCTTCCTYG[A/G]CAAGTTTCCCAGCG
PZA00460_8	5960482	G	A	GCCGGTGCTCTGATA[A/G]AGAANGGCGCGGTT
PZA00473_5	15080213	G	A	ATTTTGTGACGTGGA[A/G]CCCATTTTCTCGTA
PZA00495_5	90108	T	G	TGGATGCACTTCGAC[G/T]TTCATCTCCGACCT
PZA00498_5	16260026	C	A	GAAACAGTGGTACAA[A/C]TACGCCACCAACAG
PZA00508_2	9460083	G	A	NNNNNNNNNNNNNNT[A/G]CTAGCATTCAACAG
PZA00527_10	5960107	T	C	ACACTCCACTGTAGT[C/T]TAAGGACTGTCAAG
PZA00566_5	9460033	G	A	TTTCGATCTTGGATC[A/G]GCATATATCACCTT
PZA00571_1	9030457	G	A	GACTGTTGATCTTAT[A/G]GGGGGAGGCTCCCG
PZA00613_22	5960077	T	C	ACACCGTCAGCTTCA[C/T]GGAGATGCGAGACG
PZA00636_7	90163	G	A	GTATACATAAGAGTT[A/G]ATCGAACTGACAAG
PZA00637_6	9030671	G	A	GCCGNNNNNNTTTGA[A/G]CATACCTCTAGTGG
PZA00643_13	90193	T	G	TAACTTAGGCAGGAT[G/T]TGCATGGCATGCCA

SNP ID	SNP No.	Allele Y	Allele X	Sequence
PZA00667_2	9460096	G	A	GAACGTTGATCTCTG[A/G]GCACACTAATAAAT
PZA00680_3	9460063	C	A	TTACATGTAGCATTT[A/C]CCTGTGCCARATGG
PZA00695_3	90275	G	A	CAGTATTCAAACTGC[A/G]CTCCAGGTCAGGTC
PZA00708_3	9030306	G	C	TTTAACACTGATGAG[C/G]CAGACCAACACGGA
PZA00726_10	9460119	C	A	CAAGGTTCCAATACC[A/C]CGACGTCAAGAGCA
PZA00760_1	90308	G	C	GTAGTTTATACAGTT[C/G]AGGTGTTGTTGACA
PZA00793_2	9030581	C	A	AGTGTACAAAAAGCC[A/C]ATTGGGAATGTTCA
PZA00795_1	9030129	G	A	AAAATCCTTGGGCGG[A/G]ATACTGAAGATCTT
PZA00803_3	90112	T	G	CTTAAATGCTCCTCG[G/T]CCACATTARCTACT
PZA00814_1	9030226	C	A	CCATCACCAAGAACA[A/C]CTAGCTAGTCATCC
PZA00832_1	5960368	G	C	CCTCTGTTTTATGCA[C/G]TTTTTACAGAGTCG
PZA00856_2	13590333	G	A	CTTTCTACGGGGCAC[A/G]CATGACTAAAATAT
PZA00860_1	90342	C	A	TTATGTAATGGGTWT[A/C]AGCAGGATTAATCA
PZA00866_2	9030061	G	A	TGGTAGAGGAATCTG[A/G]TGACTGGCTGCGGT
PZA00878_2	90166	G	C	GGAGCTCACCAAAAC[C/G]TCTGTGCACACCTT
PZA00892_5	9030542	G	A	GGGGCTGTACATGCA[A/G]CTCTCTGCATATTT
PZA00941_2	15080216	G	C	AAAGCGTACTGATGY[C/G]TGTCTAAGTATTGT
PZA00942_2	15080217	C	A	CTTGAAGCTCCTGGA[A/C]AGGAAATTAAACGC
PZA00947_1	90343	T	G	TACCGTGCTCGCTAT[G/T]CGCCTGTCTTGCAG

SNP ID	SNP No.	Allele Y	Allele X	Sequence
PZA00980_1	9030470	G	A	TCTGTGAACATAGAC[A/G]TTGTCATCGACTGG
PZA01019_1	8170057	G	C	GGTCGTGGAGGACGG[C/G]TACGAGTTCTTCGC
PZA01029_1	90256	T	C	AATTTGACGTTCTTA[C/T]TGTTTATCGGTTCT
PZA01044_1	90277	G	A	GCTTATCTACGCTAC[A/G]CAGTTTCTGCGGAT
PZA01049_1	9030288	G	A	ATGTACCACCCTTTT[A/G]CGATAACTGGGCAC
PZA01062_1	90344	C	A	GGTCTTGGATAATAA[A/C]AACACCAGCAAAGG
PZA01096_1	90345	T	G	ATTCTTTGGACAAGA[G/T]GTATACTATTACAG
PZA01210_1	9460175	G	A	GATCAAAACAGCAGT[A/G]CAGTTATCCTTGCA
PZA01232_1	9030236	G	A	GGAGTGAAAGTGGAG[A/G]TTGTTACGATCAAG
PZA01254_2	15080219	G	A	GACCTGACTTGATTG[A/G]TCGGCAGTCTGCAG
PZA01267_3	9460018	G	A	ACCACTCTGGAGAAA[A/G]MRAAWNNTCAGTTC
PZA01294_1	9030658	G	C	CGAGGCGCGCCAACA[C/G]GAGCAATTMGCAGA
PZA01313_2	5960374	G	A	AAGTACGGTTGCAAA[A/G]CAAGAATTTGACTA
PZA01349_2	90204	G	A	GTAAAAGGACAGGAA[A/G]ACAGTTTTCGTGTA
PZA01374_1	9030397	G	A	ATGAGGTTATACGTG[A/G]GCWATCTGCTTAAT
PZA01427_1	90207	C	A	ACTTTTTCAATATKG[A/C]AGATTGAAGAGCAG
PZA01468_1	5960010	G	A	CCACTCTGGTTTCTG[A/G]TGGCTAAAACATTC
PZA01477_3	90561	T	C	GTCAACTAGAGTAMT[C/T]GTCGTGGTTCAATG
PZA01533_2	15080220	G	A	TGTTGCATTTGAGCT[A/G]YTRCGCTAGTTAAT

SNP ID	SNP No.	Allele Y	Allele X	Sequence
PZA01542_1	90280	G	A	CGATTATGTTGAAGG[A/G]TTATCTTTGCTCCA
PZA01601_1	9030499	C	A	AGACGTATATATASC[A/C]ATGGAAATAACAGA
PZA01607_1	9030562	G	C	GAAGCGAGTACAATT[C/G]ACACAGCCATCTGG
PZA01642_1	9030229	G	A	TTGCGATGATGGAGA[A/G]GCTAGACCACGTGG
PZA01672_1	9460160	G	A	CATTAGTTGAGGCAY[A/G]GGTCCTAATCTGAA
PZA01691_1	9030713	G	A	TCATCTGCGCCCAGA[A/G]TGTGGAGCTTCTAA
PZA01693_1	9460156	T	A	TTCAGCTTTTGTTGT[A/T]TCTCATGTTCTTTT
PZA01715_1	9030400	G	A	CCAATCCAAGCTGGG[A/G]TAGGCAAAGTAGAG
PZA01744_1	9030514	G	C	GTGGCGGCAAGGGGA[C/G]GAGAGAATGTGTCA
PZA01755_1	16260005	T	A	TATTATTATAGCAGC[A/T]TATCTGTGTGCTAC
PZA01799_1	9030512	G	A	ACGAAAAATACACKG[A/G]TTGCGGTCGGAATG
PZA01857_1	9030181	G	A	CCCAAAGCTGCTGAA[A/G]TTTGTTGATCCAAC
PZA01866_1	5960358	C	A	TGAGCTAATCNAATC[A/C]TCCTCCTGCTGTAC
PZA01885_2	90117	G	A	TGGCCTCTTATCATC[A/G]CGAGCCTCAACACG
PZA01887_1	90215	G	A	GCCAGCAGAGCTAGC[A/G]GAATCCTGCAGATC
PZA01919_2	9030452	G	C	GTGATCAACATAAAA[C/G]CATCCATTCTTGTA
PZA01933_3	9030684	G	A	CAAAGTCACCAGTGC[A/G]GTGCTAACCATCAT
PZA01962_12	15080214	T	A	AAACCCAGTAGCGTA[A/T]ACCTGCTGATTATG
PZA01972_14	9030509	G	C	AACCAAGTTCAGCGA[C/G]GTTCTTGTTCTGAG

SNP ID	SNP No.	Allele Y	Allele X	Sequence
PZA01991_3	15080221	G	A	CCCAGCAATTCTTAT[A/G]CGCCTTGATATCAT
PZA02011_1	9030568	G	A	TTAGYGKGTGCTGTC[A/G]TTTTCTATGCATCA
PZA02027_1	90555	T	C	AACTGTGAAATCCTA[C/T]ACCACCCACATCCA
PZA02058_1	9030652	T	A	TGTACTATTGAACTA[A/T]GATTATGTAATTTC
PZA02090_1	9030446	T	A	TCTATGGCAGTAAAA[A/T]TCTGCAAGCTGTTT
PZA02094_9	9460055	T	A	ACAAAGTCTCRCAAT[A/T]AGTACAAAATATAT
PZA02117_1	5960055	G	A	ATGCCTGTCATGTTG[A/G]TGATTCCCCTGGCT
PZA02164_16	9030138	G	A	CTTGGCCAAGAAGGT[A/G]GCAATGTCGTGRAG
PZA02168_1	90101	T	C	CAGTAGGYAAGACGT[C/T]TGCTCACCTGCATA
PZA02174_2	5960309	T	G	TGTTAAAGGAGCTTA[G/T]ATCAATTTAAGGAG
PZA02186_1	9030650	C	A	AAATGATACATTAGT[A/C]GTCTCCCCAGTGAT
PZA02187_1	9030453	C	A	GCAGGTAACCTGTAR[A/C]AGTTCCCTCTCACG
PZA02197_1	90350	T	G	CATTGTTGTCCTTCT[G/T]TTCGTGCAGTGCTG
PZA02207_1	9460131	G	A	TCGAAAGATGTTTCA[A/G]CCTCCCATCTGCTA
PZA02212_1	90139	G	A	AGGTGTCCCTCGAGA[A/G]ACAGCAATATGGTT
PZA02247_1	5960255	G	A	GGGCCTATAGAGATG[A/G]TTGTACATTTTGTC
PZA02269_3	5960060	T	C	GAATTCTTGCTGGAT[C/T]CTGTGTGAATCCTC
PZA02281_3	9030711	C	A	RAGCNNNNNNNNNNN[A/C]CATTTTTTATTGTA
PZA02289_2	90581	T	C	TGATAACCTGGGAAA[C/T]CAATGGCCTGTCAA

SNP ID	SNP No.	Allele Y	Allele X	Sequence
PZA02299_16	90137	G	A	GAATAAGCTGCTGCA[A/G]ACTAGAGCCATTAT
PZA02325_4	9030718	T	A	CATGTACATATANTA[A/T]TAATAGTTGGAACA
PZA02371_6	5960093	C	A	GATCTTTGGCATCCA[A/C]TCGTGCACGATGAG
PZA02373_1	9030462	G	A	CGCCTATATTTTCTT[A/G]ATGATCTGAACGAC
PZA02378_7	9030704	G	A	TGACTGCGACAGGGA[A/G]GGAGGTGCACTTCG
PZA02388_1	9030094	G	A	TGCATACTGCGGTCA[A/G]AAAACGCTCAAAGC
PZA02402_1	90140	T	C	TGATCGAAACGATTC[C/T]GTTGCCCTCGGTGA
PZA02418_2	9460073	G	A	GTGCTTGCTGGAAAC[A/G]CTAATGGATGACAC
PZA02423_1	90152	C	A	ATATGGCGTCGTATA[A/C]GCTTTATTGAGAAC
PZA02457_1	90494	G	A	GGAAGTGGAYAAGGA[A/G]CAGCAGAAGATAGC
PZA02462_1	90226	C	A	TGACAATTGTGTCCC[A/C]CGCATCTTACACAG
PZA02465_1	9030538	G	C	TAATATTCTGTGATA[C/G]GATGAAARCTTGTA
PZA02478_7	9460167	G	A	AGTTTGCGCATTCAA[A/G]TCTCGACATGCCCT
PZA02480_1	90227	G	A	TCTTCCTCTTGATTC[A/G]YAGCAACCTCAACC
PZA02496_1	9030068	C	A	GAATTACTGAAGACA[A/C]GTAATAAGTTCAGA
PZA02509_15	9030747	G	C	GAGGTAGTACAGCAT[C/G]GCGCCGGGCAGTAG
PZA02589_1	9030335	G	A	CACATCAGTTTCCCC[A/G]TCAGCCAAAGCAGG
PZA02614_2	9030521	G	A	GGGCCATCTCTTCTT[A/G]GACTTCCTCGCTCG
PZA02616_1	9460107	G	A	TGCTGAACTGGAGCA[A/G]GAAACTGTCAAATG

SNP ID	SNP No.	Allele Y	Allele X	Sequence
PZA02673_1	9030351	G	A	CAACTAATGGGTGCA[A/G]ACTTATCTTTCTTT
PZA02688_2	5960504	C	T	CTTTATGGTTGGTGG[T/C]AGAGTTTAAGCCTA
PZA02698_3	90079	G	A	ATCTCTTCTGGCATY[A/G]ATCTTATAGTTGTA
PZA02727_1	9460057	G	A	GAGTGATTTACAGCA[A/G]ATGCCCGAAGATCT
PZA02741_1	90056	T	C	CTAATCATCTGAGGC[C/T]AGYGAGGAAGAAGG
PZA02746_2	90324	T	G	TGTTCACAGCCTTCC[G/T]TGGGTTTTTGAGAG
PZA02769_1	8170053	T	A	ATCCATATATCTGCC[A/T]GTTTAGTGTTAGCA
PZA02779_1	9460118	G	A	TTCTCTACAGACTGG[A/G]TGATCTCTGCCGTG
PZA02820_17	90236	G	A	TTTTGTTATTCAGYG[A/G]CATAACAGCTATTC
PZA02854_13	9460171	G	A	GGCCTGGAGCGATGC[A/G]TGTACGAGTACGAT
PZA02872_1	9030322	G	A	CCATCGTGGGTGCCA[A/G]GAAGGACGAGCTCC
PZA02955_3	9030736	C	A	ACTGTCTCCGTGATC[A/C]ACTGCGGCACCTAA
PZA02961_6	9030616	T	A	GTTGGCGGATTCCAA[A/T]CTGGGGCAAGATCA
PZA02969_9	90392	T	C	CGCTCGGCTGACTCT[C/T]GCGTGAAGTCGTCG
PZA03020_8	9030599	T	A	TGCAAGCAAACTTGA[A/T]GTGCTGTGGTTAAA
PZA03064_6	9460050	G	A	CCTTTAATATTCGGC[A/G]TGTACCGTGGATAT
PZA03070_9	9460103	C	A	AAAATGGTGTCCCTC[A/C]CCCTTGGACCCCTA
PZA03092_7	90241	T	C	AAGAATACAAAAACA[C/T]AGCAATGCTGCCTA
PZA03116_1	5960177	G	A	TGCATATGCGTNCAC[A/G]AATGCATGCAGATG

SNP ID	SNP No.	Allele Y	Allele X	Sequence
PZA03154_4	5960150	T	C	GGAGGCCCTCATCGT[C/T]GAGCTCCACAGGGT
PZA03166_1	5960288	G	A	TAAGCTGCAGAACTC[A/G]GACACATATGCACC
PZA03167_5	90242	T	C	GACCATCTGGTTTGA[C/T]CATRRCAACAGTTG
PZA03182_5	15080227	G	A	TCCGGAACGCATTGT[A/G]CGTACCTGTTCCCC
PZA03194_1	9460023	G	A	ACGTAGAGGCCGACC[A/G]GCCGGCCAGGTTGA
PZA03200_2	9460006	G	A	TACAAATACCATACG[A/G]TCTCGGTGCAGTGA
PZA03205_1	90422	G	A	CCAGCTACCATGTCC[A/G]TTCTTCTGGATGTA
PZA03211_6	9030118	G	C	GATGGATATGATCGT[C/G]GAGCGCACGCAGGG
PZA03247_1	16260006	G	C	TCACTATCAGTAATT[C/G]AGTAGCATGTAAGT
PZA03322_5	9460124	G	C	CACAAATCTGAATTT[C/G]ATCAAACCTCTTTA
PZA03339_2	9460148	C	A	GGTACCTTCTAATAA[A/C]AAGCCAGAAATTAA
PZA03363_1	9030653	G	A	GATTTACTTGAATGT[A/G]TAAAAAATACAGTG
PZA03391_1	9030591	G	A	TCGCCAGTCGCCAAG[A/G]CTTGACARCAAGCG
PZA03409_1	90179	C	A	GCTCATCAATTTCTG[A/C]TTTGGTCTGAACAC
PZA03527_1	90127	G	A	CATTGCTGCTATGAG[A/G]AGGGAACCTAAAAT
PZA03536_1	9030454	G	A	TATGGAGATACCTMC[A/G]TGCACAAAGGGTTG
PZA03564_1	90537	T	G	AGCCAGTGTCATTCA[G/T]ATCCCATAGCAATA
PZA03578_1	90243	G	A	GCCCCCTGCAGAGAA[A/G]ACTGTTGAGAAAGA
PZA03583_1	90290	G	A	AAGTGTCTCATCGVC[A/G]AAAGAAGCTTCTTC

SNP ID	SNP No.	Allele Y	Allele X	Sequence
PZA03597_1	90182	G	A	ACGAACKCTGGGCTT[A/G]TGTTTGAGGACAGC
PZA03603_1	90393	T	A	GTAGCTTGTATTAAG[A/T]GGTTAACAGTTAGT
PZA03605_1	9030193	G	A	ATAAGTTTGACACTT[A/G]TAAAATGTGCATGT
PZA03644_1	9030516	G	A	CCATGATCTGCATTG[A/G]TGAACATGATACAT
PZA03645_1	90292	T	C	ATAATGTTTCAGTGC[C/T]TATCTGATTGACGA
PZA03647_1	9460101	C	A	GTTTCTCTTCTCGCA[A/C]GCGTTTCGTCTCTC
PZA03651_1	9030405	C	A	TACCTGGAATTAACA[A/C]AGCAAATAACATGA
PZA03670_1	90489	T	G	TGCTAGATTGTGTGC[G/T]TTACCTCATCGTTT
PZA03713_1	15080232	T	A	TTGGTCCTGTCTTTA[A/T]TGATAGCTGCGAAA
PZB00114_1	9460044	G	A	ATTCAGACAATATAG[A/G]ACTTACCACTGTTC
PZB00221_3	15080233	G	A	TGGRCATATGATGAC[A/G]TGTGCTACTAATTT
PZB00409_1	9030075	G	A	CAAATTTGATCTAAA[A/G]CTGAGGCATTCGGT
PZB00761_1	90361	T	C	AGTGCGGCTCAAGTA[C/T]GTGAAGCTGGGGTA
PZB01009_1	9460166	G	A	CGTGCGCAAGTAGCC[A/G]TCCTCCCAATGTCA
PZB01009_2	9030183	C	A	AGAGAATGAATAAAG[A/C]GGCAGTCTTTGTGC
PZB01062_3	9460034	G	A	AAATCCAGAGTTGTG[A/G]TCTTATATACTCCA
PZB01103_2	5960512	G	A	CGTGATGCTGTCACC[A/G]AACTTCAGCAGGCA
PZB01109_1	9030434	G	A	TGTGGATAATTGGTG[A/G]GTGGTTTATTTGCT
PZB01111_8	5960384	C	A	ACAGCCTGCTTGCAT[A/C]GAACCTACTTCAGC

SNP ID	SNP No.	Allele Y	Allele X	Sequence
PZB01227_6	9030638	T	A	NNNNNNNNNNAGAAT[A/T]GAATAGAATAGAAT
PZB01358_1	15080234	G	A	AAATGCATATCAACG[A/G]AAGAACAATCTCTT
PZB01403_1	9030205	C	A	TTCCACCTGTGATGA[A/C]ATTTCCAATCATGT
PZB01454_1	9030664	G	A	NNNNNTTACAGNNGA[A/G]TCTCAAATGGACTG
PZB01658_1	9030250	T	A	CCGAGGATAATCGAA[A/T]CAATTCGCGTGGAT
PZB02058_1	5960524	C	T	ATTCCACAATGACTT[T/C]TGCCCTCCTTTTGA
PZB02155_1	9030182	C	A	CATGATGCATGGCTG[A/C]CATATCTGGCGTCC
PZD00022_5	9460066	C	A	TATAACYAGGAATCT[A/C]TTCTTCTGTTTGCT
PZD00036_2	9030326	T	A	TGATGGTTCMGATAT[A/T]TCCTTGAAGTTGTC
PZE0186065237	9031042	C	T	TGATACCATCCCCAA[T/C]CACGCACTGCGTTC
sh1_12	9030432	G	A	CATGTCTGCTCCAGG[A/G]GAGACAATGTTGAA
vdac1a_1	9030504	G	C	ATAATAAATGAACAT[C/G]TTGGAGGTCCATTT
ZHD1_1	90368	T	C	CACAAGAAAACATCC[C/T]CRTCCTCTTCCTTT

Table 4.8: Grain yield, grain moisture, silking date and anthesis date ranking of the 50 inbred families at Ukulinga.

Inbred	GY rank	GM rank	SD rank	AD rank	Av. rank
15MAK8-1	34	6	1	2	3
15MAK8-74	7	12	2	1	5
15MAK9-16	21	11	3	4	6
15MAK8-31	15	9	6	8	8
15MAK8-99	14	19	4	5	9
15MAK8-59	16	16	9	3	9
15MAK8-11	19	2	12	15	10
15MAK8-130	12	13	5	12	10
15MAK8-14	35	17	7	6	10
15MAK8-135	44	3	20	10	11
15MAK8-17	22	4	15	16	12
15MAK8-19	10	5	14	19	13
15MAK8-32	45	1	23	14	13
15MAK8-4	23	24	8	7	13
15MAK9-12	4	22	10	9	14
15MAK8-33	33	7	26	18	17
15MAK8-104	5	25	11	20	19
15MAK8-5	27	39	13	11	21
15MAK9-5	2	21	18	28	22
15MAK8-85	42	10	31	26	22
15MAK8-81	39	41	16	13	23
15MAK9-6	38	18	25	32	25
15MAK8-7	32	40	19	17	25
15MAK8-125	43	8	39	31	26
15MAK8-78	49	33	24	21	26
15MAK8-35	11	37	17	29	28
15MAK8-57	6	28	30	30	29
15MAK8-84	9	34	21	33	29
15MAK8-23	47	31	34	24	30
15MAK9-27	48	29	40	22	30
15MAK8-91	18	15	33	45	31
15MAK8-88	29	23	29	41	31
15MAK9-10	3	35	32	27	31
15MAK8-93	28	48	22	25	32
15MAK9-7	46	32	43	23	33
15MAK8-97	31	36	28	37	34
15MAK9-14	41	14	45	43	34
15MAK8-76	50	43	27	34	35

Inbred	GY rank	GM rank	SD rank	AD rank	Av. rank
15MAK8-58	36	20	36	49	35
15MAK8-46	26	26	35	48	36
15MAK8-45	20	30	41	42	38
15MAK8-105	24	38	44	36	39
15MAK8-129	25	47	38	35	40
15MAK8-89	13	45	37	39	40
15MAK8-30	37	27	49	47	41
15MAK8-103	30	42	47	38	42
15MAK8-141	40	46	42	40	43
15MAK8-144	17	44	48	44	45
15MAK8-75	1	49	46	46	47
15MAK8-90	8	50	50	50	50
Average	**25.5**	**25.5**	**25.5**	**25.5**	**26**

Table 4.9: Grain yield, grain moisture, silking date ans anthesis date ranking of the 50 inbred families at Cedara.

Inbred	GY rank	GM rank	SD rank	AD rank	Av. rank
15MAK8-59	43	2	6	5	4
15MAK8-14	42	12	4	4	7
15MAK8-17	22	14	5	3	7
15MAK8-4	41	5	9	11	8
15MAK8-99	12	23	2	1	9
15MAK9-16	20	13	8	9	10
15MAK8-135	16	18	7	6	10
15MAK8-32	21	10	15	13	13
15MAK8-74	5	34	3	2	13
15MAK8-5	34	9	14	16	13
15MAK8-104	25	21	10	12	14
15MAK8-130	28	6	20	19	15
15MAK8-129	47	7	21	17	15
15MAK8-33	19	24	16	8	16
15MAK8-23	31	26	12	14	17
15MAK9-27	38	15	22	18	18
15MAK8-81	14	40	11	7	19
15MAK8-58	50	1	32	25	19
15MAK8-31	7	31	13	15	20

Inbred	GY rank	GM rank	SD rank	AD rank	Av. rank
15MAK8-35	45	16	17	26	20
15MAK8-103	49	11	1	48	20
15MAK8-88	29	4	26	32	21
15MAK8-11	33	3	33	28	21
15MAK9-12	37	19	30	23	24
15MAK8-1	32	49	18	10	26
15MAK9-10	18	20	23	36	26
15MAK9-7	48	8	41	30	26
15MAK8-125	3	35	19	27	27
15MAK9-6	26	27	35	21	28
15MAK8-78	46	36	27	22	28
15MAK9-5	23	25	28	33	29
15MAK8-19	11	42	29	24	32
15MAK8-7	44	41	34	20	32
15MAK8-84	30	30	38	29	32
15MAK8-45	24	37	25	39	34
15MAK8-141	39	28	37	37	34
15MAK8-91	8	29	31	43	34
15MAK8-46	40	17	45	44	35
15MAK8-75	6	22	42	45	36
15MAK8-57	1	48	24	38	37
15MAK8-76	10	39	36	40	38
15MAK8-93	13	45	39	31	38
15MAK8-30	35	38	43	35	39
15MAK8-97	17	47	40	34	40
15MAK8-85	15	32	46	46	41
15MAK8-105	36	43	44	42	43
15MAK8-144	4	33	49	50	44
15MAK8-89	9	44	48	41	44
15MAK8-90	2	46	47	49	47
15MAK9-14	27	50	50	47	49
Average	**25.5**	**25.5**	**25.5**	**25.5**	**26**

Table 4.10: Grain yield, grain moisture, silking date and anthesis date ranking of the 50 inbred families across the two sites.

Inbred	GY rank	GM rank	SD rank	AD rank	Av. rank
15MAK8-17	20	6	4	4	5
15MAK8-59	22	4	6	5	5
15MAK8-14	41	11	3	3	6
15MAK8-99	12	18	2	2	7
15MAK9-16	18	10	5	7	7
15MAK8-4	14	8	7	10	8
15MAK8-135	42	9	11	8	9
15MAK8-74	5	28	1	1	10
15MAK8-32	37	3	15	13	10
15MAK8-130	17	5	14	17	12
15MAK8-33	28	13	18	11	14
15MAK8-104	7	19	10	14	14
15MAK8-31	11	23	9	12	15
15MAK8-5	30	17	13	15	15
15MAK8-11	23	2	21	23	15
15MAK9-12	9	14	17	18	16
15MAK8-1	32	37	8	6	17
15MAK8-35	24	20	16	29	22
15MAK8-81	31	46	12	9	22
15MAK8-23	45	32	19	16	22
15MAK9-27	48	16	34	20	23
15MAK8-19	13	31	20	21	24
15MAK8-88	29	7	29	37	24
15MAK9-5	3	27	22	28	26
15MAK9-7	49	12	41	24	26
15MAK8-58	47	1	36	42	26
15MAK9-10	6	22	28	31	27
15MAK8-78	50	36	24	22	27
15MAK9-6	34	26	30	27	28
15MAK8-129	36	30	32	25	29
15MAK8-7	38	45	23	19	29
15MAK8-84	16	33	25	32	30
15MAK8-125	25	29	31	30	30
15MAK8-57	2	38	26	33	32
15MAK8-93	21	49	27	26	34
15MAK8-103	43	21	39	43	34
15MAK8-46	33	15	42	47	35
15MAK8-91	15	25	35	45	35
15MAK8-85	35	24	43	41	36
15MAK8-76	46	44	33	34	37

Inbred	GY rank	GM rank	SD rank	AD rank	Av. rank
15MAK8-45	19	35	38	39	37
15MAK8-97	27	40	37	35	37
15MAK8-141	44	42	40	38	40
15MAK8-105	26	41	44	36	40
15MAK8-30	39	34	47	46	42
15MAK8-89	10	48	46	40	45
15MAK8-75	1	43	45	48	45
15MAK8-144	8	39	49	49	46
15MAK9-14	40	47	48	44	46
15MAK8-90	4	50	50	50	50
Average	**25.5**	**25.5**	**25.5**	**26**	**26**