# Genetic characterization of pro-vitamin A and quality protein maize inbred lines and their derived hybrids

by

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# GENERAL ABSTRACT

Maize is one of the most important crop plant, valued both as cereal and forage crop because of high nutrition and palatability. Sub-Saharan Africa (SSA) countries are highly dependent on maize compared to other African countries and there is preference of white maize over orange maize, which leads to Vitamin A deficiency (VAD) crisis. Vitamin A deficiency can be alleviated by increasing pro-Vitamin A maize consumption rate in the population. In orange maize, Vitamin A is in the form of pro-Vitamin A, therefore crops with high content of pro-Vitamin A carotenoids are a promising strategy to alleviate Vitamin A content among disadvantaged populations. Lack of quality proteins in maize is another challenge faced the consumers of this staple crop in SSA. Therefore maize requires improvement in this regard. Quality protein maize (QPM) was developed from mutant maize with an opaque- 2 gene that improves amino acids; lysine and tryptophan. Lysine and tryptophan allow the body to manufacture complete proteins, and tryptophan lessens the prevalence of kwashiorkor in children. Studies clearly show that QPM could be superior to normal maize (NM) if used in the diet of humans. The objectives of this study were therefore to analyze the genetic diversity among the Pro-Vitamin A lines, Quality Protein Maize lines, and Normal Maize lines; to identify potential heterotic groups, and to evaluate their $F_1$ hybrids. Twenty maize inbred lines were used in the study, comprising 13 Pro-Vitamin A; four QPM and three normal lines. The lines were genotyped with 93 SNP markers at the DNA Landmarks Laboratory in Canada. Data was analyzed using the PowerMarker version 3.25 statistical package. The hybrids were generated in a 4 X 10 North Carolina design II with reciprocal mating which resulted in 78 experimental hybrids with adequate seed for planting in trials. Three commercial hybrids were included as controls. The 81 hybrids was evaluated in a 9 x 9 alpha-lattice design with two replications at four sites in KwaZulu-Natal, South Africa. The data collected was analyzed using Genstat. Out of 93 SNPs markers used, six of them were monomorphic and 87 polymorphic. The use of SNP markers was effective; the data set reflected the homogenously homozygous state of inbred lines and was able to determine the genetic diversity and distance. Inbred lines that showed the highest genetic distance were normal maize (DPVA17) and pro-Vitamin A (DPVA12) which was 0.54; and lowest genetic distance was observed between normal maize (DPVA19) and normal maize (DPVA18) which was 0.11. In the current study the higher genetic diversity was observed

between previously identified groups and six potential heterotic groups were identified. Grain yield of the hybrids was highly significant at Cedara and Dundee, and not significant at Jozini and Ukulinga. The three economic traits (ear aspect, number of ears per plant, and grain moisture content) were significant at all sites. Genotype x environment interaction effects were observed. Performance of hybrids varied with sites. At Cedara, the highest performing experimental hybrid was 14PVAH-29, Dundee; 14PVAH-166, Jozini; 14PVAH-8, and Ukulinga; 14PVAH-50, respectively. Hybrid 14PVAH-139, 14PVAH-129, 14PVAH-149, and 14PVAH-10 were placed by three methods in top 10% stable hybrids, thus they are considered as the most stable hybrids. The current study showed that traits such as number of ears per plant, plant height, and ear aspect are strongly associated positively with yield, and that stem lodging, root lodging, and ear rot are negatively associated to yield. Although DNA molecular markers can be used in identifying heterotic groups, the relationship of genetic distance and hybrid vigour is still not well understood. Therefore, both molecular markers and conventional field trials (phenotyping) must be used to identify heterotic groups among pro-Vitamin A and quality protein maize. This would be crucial for devising breeding strategies for developing nutritionally rich maize hybrids.

# DECLARATION

I, Lindokuhle Phakathi declare that:

I. The research reported in this thesis, except where otherwise stated is my original research.

II. This thesis or any part of it has not been submitted for any degree or examination at any other university.

III. This thesis does not contain any other person's data, pictures, graphs or other information, unless specifically acknowledged as being sourced from those persons.

IV. This thesis does not contain other persons writing, unless specifically acknowledged as being sourced from them. Where other written sources have been quoted, then
   a. their words have been re-written but the general information attributed to them has been referenced.
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Signed: _____

Lindokuhle M. Phakathi (candidate)

Signed: _____ Signed: _____

Professor John Derera (Supervisor) Dr. Edmore Gasura (Co-Supervisor)

# DEDICATION

I would like to dedicate this work to God who made everything possible, and carried me through all the challenges and my supportive and loving mother Nonhlanhla Phakathi.

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# LIST OF ABBREVIATIONS

AIMRD: Abstract, Introduction, Materials and Methods, Results, and Discussion

ANOVA: Analysis of variance

AMMI: Additive main effects and multiplicative interaction

ASI: Antheis-silking interval

CIMMYT: International Maize and Wheat Improvement Center

CV: Coefficient of variation

DNA: Deoxyribonucleic acid

EH: Ear height

EPO: Ear position

EPP: Number of ears per plant

ER: Ear rots

FAO: Food and Agriculture Organization of the United Nations

FAOSTAT: Food and Agriculture Organization Statistics

GD: Genetic distance

GE: Genotype by environment interaction

IPCA: Interactive principal component axis

LSD: Least significant difference

MAS: Marker-assisted selection

PCR: Polymerase chain reaction

PH: Plant height

PIC: Polymorphic information content

RAPD: Randomly amplified polymorphic DNA

REML: Restricted maximum likelihood

RFLP: Restriction fragment length polymorphism

RL: Root lodging

SE: Standard error

SL: Stem lodging

SNP: Single nucleotide polymorphism

SSA: sub-Saharan Africa

SSR: Simple sequence repeats

UKZN: University of KwaZulu-Natal

UPGMA: Unweighted paired group method using arithmetic averages

USA: United States of America

VAD: Vitamin A deficiency

# CHAPTER ONE

# GENERAL INTRODUCTION

## 1.1 Importance of maize

Maize (*Zea mays* L.) is a widely grown grass plant which originated in Mexico about 6,000-7,000 years ago (Mangelsdorf and Galinat, 1964, as cited by Lee, 1994). It is one of the most important economic crop plants, valued both as cereal and forage crop; because of high nutrition and palatability (M'mboyi et al., 2010). Maize is an important source of carbohydrates and protein. In East and Southern Africa, about 15.5 million hectares are planted with maize annually. However, maize is the staple crop for 24 million households, and this serves as an indication that per capita consumptions are higher than what is being produced. M'mboyi et al. (2010) reported that more than 200 million people in sub-Saharan Africa (SSA) face widespread severe food shortages. Furthermore, Shiferaw et al. (2011) reported that the yields of maize are exceedingly low (not more than 1.5 t $ha^{-1}$) in poor countries in Africa and Latin America which are dependent on maize.

M'mboyi et al. (2010) reported that it is predicted that by 2020 maize will surpass wheat and rice to become the number one most grown cereal. This is because of climate change, since maize is adapted to wide range of environments. Furthermore it is more genetically diverse than any other cereal crop (Flint-Garcia et al., 2009) due to its high open pollination potential. Nonetheless, maize productivity improvement is under great challenge in SSA due to low inputs, poor mechanized agricultural practices, and notably poor yielding varieties. Environmental conditions such as drought stress, are also among the major constraints contributing to yield losses (Diallo et al., 2004) . Therefore genetic improvement of SSA maize varieties by both public and private sector is in demand.

## 1.2 Improvement of maize yields

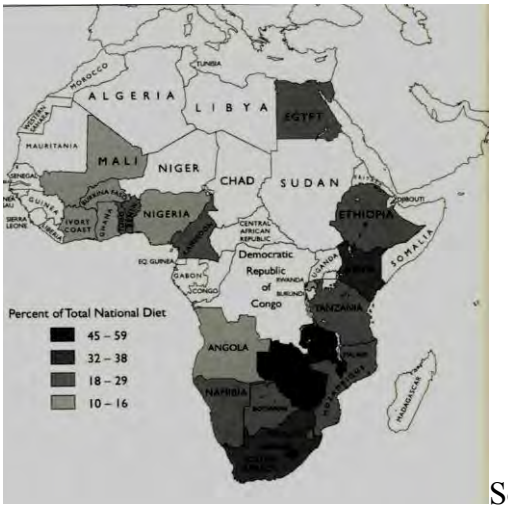
Many interventions have been implemented to improve maize yields such as the study of genotype by environment interaction (GE) and use of molecular markers. Environmental conditions can be influenced by factors such as low soil-fertility, pest and disease pressure, and drought which results into major implications by reducing response to selection however, through multi-location and multi-seasonal testing GE can be quantified (Babic et al., 2011). GE refers to the differential response of varieties grown in the environment. Different molecular markers have been used in maize to screen large populations and for trait improvement. The latest single nucleotide polymorphism (SNP) marker is currently the preferred markers among other Deoxyribonucleic acid (DNA) based markers. SNP markers are most popular markers for fine mapping of heritable traits in maize (Laguadah et al., 2009).

## 1.3 Malnutrition and Pro-vitamin A deficiency

Africa is a leading continent in the crisis of malnutrition, and the problem of vitamin A deficiency (VAD) this is due to the heavy dependence on white maize in the diet, which causes Kwashiorkor. Vitamin A is a threat to human health, especially in SSA and other developing regions of Asia and Latin America. Among other effects, VAD results in night blindness, loss of appetite, poor growth rate and weakened immune system (Gibson, 2005, as cited by Pillay et al., 2011). Figure 1 shows maize consumption in different African countries as a percentage of national diet. It can be seen from Figure 1 that SSA countries are highly dependent on maize compared to other African countries. Dent, white maize is widely used for human consumption (FAO, 1992). However, the nutritional composition of white maize lacks provitamin A carotenoids (Nuss and Tanumihardjo, 2010). In plant foods, vitamin A is in the form of provitamin A carotenoids (Pillay et al., 2011), and crops such as orange maize, orange fleshed sweet potato (*Ipomoea batatas* L.) that have high content of provitamin A carotenoids are a promising strategy to alleviate vitamin A content among disadvantaged populations.

There are various interventions to supplement malnutrition, such as implemented by HarvestPlus (2009) which aims to improve food security and quality by reducing micronutrient malnutrition

amongst less advantaged populations in Africa, Asia and Latin America. Quality protein maize (QPM) was developed from mutant maize with an opaque- 2 gene that improves amino acids; lysine and tryptophan (Vasal, 2000). Lysine and tryptophan allow the body to manufacture complete proteins, and tryptophan lessens the prevalence of kwashiorkor in children (Graham et al., 1969). Studies clearly show that QPM could be superior to normal maize (NM) if used in the diet of humans to supplement malnutrition.



Source: (Pillay et al., 2011)

Figure 1.1: African maize consumption as a percentage of national diet

**1.4 Significance of the study**

In SSA, maize yields are low due to low inputs, poor mechanized agricultural practices, poor environmental factors, such as drought, and most importantly poor yielding varieties, which are poorly adapted to the environment. The best strategy to improve maize productivity in SSA is through genetic improvement of varieties to provide higher yields under unfavorable environmental conditions. Maize varieties high in pro-Vitamin A and with high quality protein have been found to contribute towards alleviation of malnutrion and VAD. Therefore these varieties must be evaluated and supplied to the farmers. This can be attained through the use of both conventional and molecular plant breeding. Molecular markers, such as single nucleotide polymorphism (SNPs), may be used to aid conventional breeding by determining genetic

diversity needed to assign inbred lines into heterotic groups and thus speed up the breeding process.

### 1.5 Research objectives

The main objective of this research was to characterize different Pro-Vitamin A and quality protein (QPM) maize inbred lines and relate the genetic observations to the agronomic performance of their $F_1$ hybrid progeny. This was to be achieved through the following specific objectives:

- To analyze the genetic diversity present among the Pro-Vitamin A, Pro-Vitamin A, Quality Protein Maize and Normal Maize lines.
- To identify potential heterotic groups among the Pro-Vitamin A (UKZN), Quality Protein Maize, and Normal Maize lines.
- To identify the top 10% hybrids in terms of hybrid stability.
- To identify traits associated with high yield potential across four environments in KwaZulu-Natal.

### 1.6 Research hypotheses

The research hypotheses were as follows:

1.6.1 There is a significant difference in genetic diversity and distance among the Pro-Vitamin A, Quality Protein Maize and Normal Maize lines.

1.6.2 There is a significant difference among inbred lines, and different lines can be allocated into different heterotic groups in accordance with their shared similarities, distances and origins.

1.6.3 There is a significant difference among experimental hybrids within and across four environments based on yield and other economic traits, and the top 10% of high yielding hybrids can be separated from all experimental hybrids.

1.6.4 There is a significant difference among traits and their association with high yield potential could be identified as negative or positive.

### 1.7 Outline of the thesis

This thesis is laid as follows:

- Chapter 1: General Introduction

  Contains the importance of maize; information on Pro-Vitamin A deficiency; the significance of the study; objectives, research hypotheses; and the outline of the thesis.
- Chapter 2: Literature Review

  The literature was reviewed based on the objectives of the present study, where the following main domains were discussed: Maize production and improvement, maize inbred lines and hybrids, heterosis and heterotic grouping, genetic diversity and estimation of genetic diversity, genetic distance, relationship of genetic distance and heterosis, background of molecular markers and different types of molecular markers, the use of single nucleotide polymorphisms (SNP) and markers in maize. Based on the literature review conclusions were drawn at the end.

- Chapter 3: Characterization of Twenty Maize Inbred Lines Using Single Nucleotide Polymorphisms Markers

  The introduction mainly focuses on genetic diversity and distance; the methods and material chapter describes plant material preparation and methods of DNA extraction and genotyping. The results presented shows all analyzed parameters and discussion, and conclusions were drawn.
- Chapter 4: Selection of Superior Hybrids at Four Different Sites and Evaluation of Association of Traits with Yield

  The introduction summarizes hybrid maize utilization, genotype x environment interaction, and genetic gains; the methods and material chapter describes the experimental designs, environmental sites and environmental management techniques, and also describes how the data was collected and analyzed. The results presented mainly focus on presenting yield and economic traits in each site, best performing hybrids and how traits associates with yield. Presented data under results were discussed and conclusions were drawn.
- Chapter 5: General Overview of the Research and Way Forward

The objectives of the study and major findings of the study were listed, and the implications and recommendations for fucture purposes are given.

Chapters 3 and 4 are written in AIMRD format, which includes: Abstract, Introduction, Materials and Methods, Results, and Discussion. All chapters comprise a reference list and the referencing style used in this thesis is according to the guidelines used for Harvard referencing style.

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# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Introduction

The literature was reviewed based on the objectives of the present study, where the following main domains were discussed: Maize production and improvement, maize inbred lines and hybrids, heterosis and heterotic grouping, genetic diversity and estimation of genetic diversity, genetic distance, relationship of genetic distance and heterosis, background of molecular markers and different types of molecular markers, the use of single nucleotide polymorphisms (SNP) and markers in maize. Based on the literature review, conclusions were drawn at the end.

## 2.2 Maize production and importance

Maize (*Zea mays* L.) is one of the most produced crops worldwide. It is ranked in 6$^{th}$ position after rice, wheat, soybean, tomatoes, and sugar cane (FAOSTAT, 2010). Maize is a major staple crop for more than one billion people in sub-Saharan Africa (SSA) and Latin America (HarvestPlus, 2009). In Southern Africa, it is the number one crop which is consumed in not less than two meals a day. Most importantly, maize is not solely used for one objective. It is used for human food, animal feed and industry (Moreno et al., 2005). Its popularity in SSA is mainly due to its ability to thrive in a wide range of soil types and many agro-ecological zones, and its ability to produce sustainable yields at minimal agronomical inputs for sustainable farmers (Akinwale et al., 2014). However, commonly attained yields are low per unit area. This is not only compromised by poor agronomic practices but also by poor genetic improvements. Thus the production does not match with the human and animal consumption rate. Worldwide, consumption of maize is more than 116 million tonnes, with Africa consuming 30% and SSA 21% (IITA, 2010). Table 1 shows that among African countries, South Africa was the leading country in maize production in 2010. This is mainly due to research inputs to provide genetically improved crops and implications of biotechnology by international companies, such as Monsanto. Furthermore, according to FAOSTAT (2010), maize was ranked number one in the

top crops produced in South Africa during 2010; followed by grapes, sugar cane, apples, and potatoes.

Table 2.1: Ranking of maize production per country

| Country | Production (Million tons) |
|---|---|
| United States of America | 273,832,130 |
| China, mainland | 208,130,000 |
| Brazil | 71,072,810 |
| Mexico | 22,069,254 |
| Argentina | 21,196,637 |
| India | 21,060,000 |
| Ukraine | 20,961,300 |
| Indonesia | 19,377,030 |
| France | 15,614,100 |
| **South Africa** | **11,830,000** |
| Canada | 11,703,100 |
| Nigeria | 9,410,000 |
| Philippines | 7,406,830 |
| Ethiopia | 6,158,318 |
| Romania | 5,953,352 |
| United Republic of Tanzania | 5,104,248 |
| Pakistan | 4,631,000 |
| Malawi | 3,618,699 |
| Kenya | 3.600,000 |
| Zambia | 2,852,687 |

Source: (http://faostat.fao.org, accessed 12 June 2014).

Maize approximately accounts for 30−50% of low-income household expenditures in Eastern and Southern Africa (ESA). The heavy dependence on maize in the diet leads to malnutrition and vitamin A deficiency which causes kwashiorkor (IITA, 2010) among other malnutrition related

diseases. In SSA, maize is consumed by 50% of the population and is the preferred food for one-third of all malnourished children and 900 million poor people worldwide. Furthermore, between now and 2050 the demand for maize in the developing world is expected to double (M'mboyi et al., 2010). This justifies research on maize yield and nutrition to be increased in SSA.

### 2.3 Importance of pro-Vitamin A maize and quality protein maize

One of the most devastating problems faced by children in SSA is vitamin A deficiency (VAD), resulting in some malnutrition symptoms (HarvestPlus, 2009). Furthermore, VAD is associated with increased infection by diseases and night blindness (Wilson et al., 1953). However, through improvement of orange maize varieties, high in Pro-Vitamin A (PVA) content the crisis of Vitamin A deficiency may be abridged or eradicated. However, studies on consumer acceptance of PVA rich (orange) maize conducted in KwaZulu-Natal (South Africa) have shown that there is a cultural preference for white maize over orange maize (Pillay et al., 2011). In this regard, most women and children are under-nourished with frequent occurrences of vitamin A deficiencies (West Jr and Darnton-Hill 2008).

Quality protein maize (QPM) was developed from mutant maize with an opaque- 2 gene that improves amino acids; lysine and tryptophan (Vasal, 2000). Lysine and tryptophan allow the body to manufacture complete proteins, and tryptophan lessens the prevalence of kwashiorkor in children (Graham et al., 1969). Studies clearly show that QPM could be superior to normal maize (NM) if used in the diet of humans (Kiria, 2010). Less than 1% of the 30 million hectares of maize production in SSA are QPM thus far, but the required agronomic practices are the same as for normal maize (Machida et al., 2014).

### 2.4 Maize inbred lines and hybrids

Maize inbred line development relies on successive inbreeding through self-pollination with selection biased by desirable traits. Maize inbred lines have provided the research division of maize, a large array of uniformity and reproducible genotypes (Lee, 1994). Usually, inbred lines

have been developed to be utilized as parents in hybrid breeding programmes. Information on pedigrees has been helpful in research for predicting characteristics of germplasm and for assigning inbred lines into heterotic groups. Heterotic grouping refers to group of inbred lines which express the similar combining ability or heterotic response when crossed with other genetically dissimilar group of inbred lines (Ornella and Tapia, 2010). Inbred lines which are genetically dissimilar have high potential of expressing high heterosis. Conversely, genetically similar inbred lines express inbreeding depression. Therefore, heterotic groups can be formed based on studying genetic diversity among inbred lines before crossing them (Fato, 2010). There are various methods used to classify inbred lines into heterotic groups (Windhausen et al., 2012).

Heterotic groups provide a convenient way of managing genetic resources, then hybrids are generated from crossing lines from different heterotic groups to maximize vigour and heterosis (Akinwale et al., 2014). Hybrid maize planting improves farmer's productivity and warrants a dependable, sustainable food supply because of hybrid vigour. It is explained by the phenomenon of heterosis which is referred as the increased agronomic performance of heterozygous $F_1$ plants compared to their homozygous inbred plants (Lee and Kannenberg, 2004). In hybrid breeding programmes, single crosses have been identified to be more efficient for developing commercial hybrids compared to double crosses (the cross between two different $F_1$ hybrids), and remains the method of choice for reaching maximum genetic gain from the effects of heterosis. Yet, selection of inbred lines to give rise to high performing hybrid is the most crucial, costly and time consuming phase in hybrid development programmes (Gissa et al., 2012).

**2.5 Heterosis**

Heterosis was coined in 1914 by Shull, and refers to the expression of superiority of $F_1$ performance relative to parental performance (Robert, 2013). There is a tendency of increase in vigour due to crossing of inbred lines. Heterosis has been used in the production of many crops, such as maize, rice, cotton, sorghum, and oilseeds; thus significantly improving production per unit area of these crops (Premlatha et al., 2011). Utilization of heterosis as an approach for crop

improvement was invented by Jones in 1917 through the development of double cross hybrids in maize. The use of hybrids in maize for production has increased significantly after Jones demonstrated this strategy. Considerable effort has been spent on using molecular markers in an attempt to understand the molecular foundations of heterosis and predict heterotic response. The potential of the approach based on genetic distance model using different molecular markers, namely, isozymes, restriction fragment length polymorphism (RFLPs), amplified fragment length polymorphism (AFLPs), simple sequence repeats (SSRs), and single nucleotide polymorphism (SNPs) for predicting heterosis has been studied in great detail in the case of maize (Roychowdhury et al., 2014). Although various studies have been conducted to explain heterosis: its genetics, physiology, molecular, and biochemical foundations are still largely unexplained (Reif et al., 2005). Therefore, further knowledge on heterosis can result in advanced exploitation of hybrids to improve yields. However, this is outside the scope of this study which only focuses on finding heterosis among the three groups of lines under study (PVA, QPM and NM).

### 2.5.1 Heterotic grouping

Identification of heterotic groups among inbreds is crucial for the success of a maize hybrid breeding programme, this is because an understanding of the genetic relationship among the inbred lines involved in a maize breeding programme has been reported to be useful in planning crosses and assigning the lines to specific heterotic groups for the purpose of developing high yielding hybrids (Akinwale et al., 2014). In a hybrid maize breeding programme, most crosses are rejected after field assessment due to low performance. Molecular markers have been identified as potential tools for predicting hybrid performance through assessing the molecular diversity between parental lines (Nikhou et al., 2013). The foundations to use molecular markers are explained by quantitative genetic theory which has shown that heterosis is a function of genetic divergence between inbred parental lines involved in a cross (Riedelsheimer et al., 2012). The use of molecular markers for genetic grouping of lines was proposed by Comstock and Moll (1963).

Pedigree and origin of inbred lines have been used successfully in several studies to allocate maize lines into heterotic groups. This is because lines from similar families are likely to be genetically similar and lines from the same origin have the same adaptation. Zhang et al. (2002) showed that cluster analysis based on specific combining ability can be used to classify inbred lines into heterotic groups. As mentioned above, molecular markers are used to assign maize inbred lines into heterotic groups (Godshalk et al., 1990). Riedelsheimer et al. (2012) successfully used the Illumina SNP chip MaizeSNP50 containing 56,110 SNP markers for genotyping a population of 285 inbred lines. The prediction accuracies ranged from 0.72 to 0.8, allowing them to reliably screen for large collections of diverse inbred lines for their potential to create superior hybrids. Other several studies indicate that grain or biomass yield of maize hybrids can be predicted with high accuracy when employing genetic diversity studies of inbred lines (Albrecht et al., 2011; Pérez-Rodríguez et al., 2012).

### 2.6 Genetic diversity

Genetic diversity refers to the variety of alleles and genotypes present in a population. It can be accessed through morphological, physiological, biochemical, and behavioral differences between the individuals within or between the populations (Dubreuil and Charcosset, 1998). Researchers significantly benefit from the information on genetic diversity of their germplasm. This is because genetic variability is essential to develop a variety (Cholastova and Knotova, 2012). Genetic diversity studies focus on the analysis of variation among individuals or groups of individuals or populations by a specific method or a combination of methods. The data often involve numerical measurements and in many cases, combinations of different types of variables.

The first report of molecular diversity used to predict the heterotic hybrid performance came from maize (Makumbi et al., 2011). Fortunately, maize is recognized as one of the most diverse crop species characterized at both morphological and molecular (Chen et al., 2011). Maize molecular diversity is roughly 2-5 folds higher than that of other commonly domesticated cereals (Flint-Garcia et al., 2009). Genetic diversity has been intensively utilized in maize breeding programmes, this is because maize is a naturally, heterozygous, out-crossing species susceptible

to inbreeding depression (Li et al., 2008). In maize hybrid breeding programme, inbred lines are allowed to cross and give rise to a genetically diverse hybrid at an allelic level that responds favorably to the environment.

Genetic diversity is imperative for maintaining production in crops. It provides genetic strength against different unfavorable environmental conditions such as drought, and disease pressure (Nataraj et al., 2014). Consequently, there are numerous studies where genetic diversity among individuals in different populations has been analyzed (Basaki et al., 2009; Li et al., 2008; Dubreuil and Charcosset, 1998; Song et al., 2013; Khierallah et al., 2013). It has been noted from several studies reviewed about understanding the basis of genetic diversity that most of them were conducted through the use of microsatellite markers. Akinwale et al. (2014) indicated that in the early 90s, PCR-based markers such as SSR and AFLP have proved to be powerful tools for analyzing genetic diversity. Chen et al. (2011) conducted a study, where the morphological and genetic diversity and population structure for 76 maize recombinant inbred lines was investigated using 48 SSR and 17 morphological traits. The authors concluded that this approach was effective for establishing marker-trait relationships, and confirmed that association mapping could complement and enhance previous QTL information for marker-assisted-selection (MAS). Molecular marker-based genetic diversity is important for genetic mapping and MAS in breeding (Lapitan et al., 2007). The improvements in DNA marker technology contributed to the process of representing diversity within plant population, crop germplasm and establishing DNA fingerprints for each genotype.

### 2.6.1 Estimation of genetic diversity

Before the development of molecular markers in early times, the assessment of genetic diversity was attained through pedigree analysis, morphological traits, physiological, and biometric analysis of both qualitative and quantitative traits (Legesse et al., 2008). However, there are limitations that can result in inaccurate information when assessed through morphological traits (Cholastova and Knotova, 2012); and pedigree (Legesse et al., 2008). Biometrical techniques available for analyzing genetic diversity, include analysis of variance, correlation, regression and

multivariate techniques. However, limitations from with the use of biometrical techniques results from random error and genotype x environment interaction (GE). Consequently, molecular markers have been the preferred method. Molecular markers such as SSRs have been adopted for the use in nowadays research characterization of genetic diversity in maize (Reif et al., 2006, Qi-Lun et al., 2008, Eschholz et al., 2010 cited by Aci et al., 2013).

### 2.7 Genetic distance

Genetic distance can be determined through molecular markers and be used to identify and group inbred lines into heterotic patterns (Legesse et al., 2008). The genetic distance based molecular marker can be applied for initial heterotic grouping of inbred lines (Dhliwayo et al., 2009 cited by Kustanto et al., 2012). Multivariate analysis is one of the major tools currently used in estimating genetic distance; additionally Mahalanobis (D2) and Euclidean distances are the most used statistics to estimate genetic diversity (Bertan et al., 2007). In the current study data was evaluated using PowerMarker version 3.25 according to Nei (1973) methodology. Classically, genetic distance analysis of maize inbred lines was based on morphological traits, however the recent studies address genetic distance analysis through molecular markers (Abed and Abed, 2013).

Numerous studies have shown that molecular markers are potentially helpful in determining the genetic distance among maize inbred lines (Abed and Abed, 2013; Yu et al., 2012; Nelson et al., 2011; George et al., 2011; Lanza et al., 1997). Single nucleotide polymorphism markers are currently the marker of choice; however, studies show that SSR markers are still intensively used to analyze genetic distance in maize. Nelson et al. (2011) studied the number, genomic coverage, and discrimination abilities of SNP markers required to provide equivalent measures of genetic distance compared to previously assayed SSR loci among maize inbred lines. The effectiveness of each SNP marker set was evaluated by comparison with standard SSR marker sets and pedigree distance values. Nelson et al. (2011) also found that, SNP markers are selected to maintain high expected heterozygosity (He) and even genome coverage. Therefore, data from

only 2 to 3 times the number of SNP markers are needed to reveal associations among lines compared with SSR markers.

### 2.7.1 Relationship of genetic distance and heterosis

A complex relationship between genetic distance and heterosis has been noted in maize (Reif et al., 2013). However, some studies show the relationship to be linear, where heterosis is largely expressed between two divergent inbred lines (George et al., 2011). Moll et al. (1965) showed that heterosis response in maize increases as the genetic distance between parent increases but only up to an optimum level. Genetic distance based on molecular markers have been extensively interrelated with heterosis in several crops such as maize, oats, rice and wheat, but with different results (George et al., 2011). However, in many cases, the estimates of correlation between GD and heterosis were statistically significant but too low to be useful for prediction.

A study conducted by Reif et al. (2003) using SSR markers and Marsan et al. (1998) using RFLP and AFLP markers concluded that there is a positive correlation between genetic distance and heterosis, but the relationship is too small to be of any practical value. This is mainly because it cannot be guaranteed that inbred lines from different heterotic groups will always give rise to high performing hybrids. This was explained by Betrán et al. (2003) who reported that the degree of heterosis is dependent to the relative performance of parents. The authors also concluded that environmental stress had an influence towards the use of genetic distance as a predictor of hybrid performance which is still yet not understood. Qi et al. (2010) suggested that heterotic breeding strategies in maize can be improved by predicting reliably heterosis; this can be achieved through correlating high specific combining abilities (SCA) in hybrid performance and a DNA marker-based genetic distance in the inbred lines. Qi et al. (2010) also used AFLP markers for genetic distance and evaluated SCA in a diallel set of crosses. They concluded that although AFLP markers can be used to detect the genetic divergences, place maize inbred lines in different heterotic pools and identify the most positive SCAs and heterosis, they are still limited in fully predicting hybrid performance.

## 2.8 Molecular markers

Melchinger and Gumber (1998) cited by Song et al. (2013) reported that the development of molecular markers has led plant breeders to be able to screen large populations of plants for crop improvement. Molecular markers are indicators that can mark specific positions along the genome (Kumar et al., 2009). However, it is costly and laborious to analyze genetic relationships among populations with large-scale molecular markers. In the same population of plants or on an individual plant the same gene may exist in different alternative forms which are referred to as alleles; thus, molecular markers may be used to identify an allele or allelic combinations expressing a desirable trait (Robert, 2013). The screening is based on the presence or absence of a certain gene as determined by laboratory procedures, rather than on the visual identification of the expressed trait in the plant.

Molecular markers are key tools for plant identification and plant improvement. Remarkable achievements have been made in crop improvement by exploring the genomes of individual crop species through the use of molecular markers. Genetic markers were initially used in genetic mapping to find out the order of genes along chromosomes. Alfred H. Sturtevant in 1913 generated the first genetic map using six morphological traits in the fruit-fly (Andersen and Lübberstedt, 2003). Molecular marker technology continues to advance, the early technologies were non-DNA-based, however, the latest technologies are DNA-based which results into improved DNA analysis (Xu, 2010). Nowadays, molecular markers are used in plant improvement, plant conservation, plant bio-security, harnessing heterosis, and genetic variant discovery of crop species (Robert, 2013). However, the main objective of the use of molecular markers in plant breeding is DNA sequencing for identifying variations at a locus (Newbury, 2003).

The use of molecular markers in plant improvement started in the late 1960s, where protein markers were developed for plant genomes. These markers were isozymes that could be visualized by staining after separating according to size (Robert, 2013). It was made achievable to show a relationship between isozymes and important agricultural traits in crop species.

However, isozyme surveys represented a basic, but fruitful level of investigation for species, but markers detecting variation directly at the DNA level were found to be more helpful in crop improvement (Dubreuil and Charcosset, 1998). The main weakness of isozymes is their relatively low abundance and low level of polymorphism (Kumar et al., 2009). The most helpful molecular marker in crop improvement is notable by ability to determine multiple alleles per locus and allows each allele to be observed (Xu, 2010). The advances towards DNA-based markers in the 1980s have been utilized to assist with breeding programmes and today breeders can use a number of molecular markers simultaneously to search for DNA markers that are associated with traits of interest (Xu and Crouch, 2008). Therefore, the efficiency and precision of plant breeding programmes can be increased through marker-assisted-selection. The capability to anticipate the hybrids between diverse heterotic groups is of fundamental importance for developing hybrids with improved performance.

Molecular markers can be exploited as predictive tools in the context of practical hybrid plant breeding; however, in the past much energy has been expended on using molecular tools in an attempt to understand the molecular basis of heterosis (Robert, 2013). One of the recent examples is provided by Shi et al. (2011) who analyzed *Brassica napus* L. population to understand the genetic basis of heterosis using molecular markers. Importantly, it was noted that heterozygosity of hybrids was not always responsible for positive impact on the performance of the hybrid.

Other fundamental uses of molecular markers such as plant conservation is to assess the long- and short-term viability of populations and species with small effective population sizes (Morin et al., 2004). In small population due to limited mating partners, plants go through inbreeding depression, loss of genetic diversity, and loss of adaptive potential. Therefore, the use of molecular markers provides prediction concerning longevity of species viability of the threatened species, to develop long-term management strategies (Robert, 2013). Molecular markers are also exploited in detecting the presence of genetic variation in germplasm collections and breeding lines. This have been employed as a methodology to verify the new variety, and resolve issues of plant breeder's right for the particular variety by verifying the varietal identity, purity and stability. However, there are general disadvantages involved from employing the use of

molecular markers to breeding programme, such as investment requirement to train people to be skilled for application of molecular markers, and conventional breeding methods can be cost-effective.

### 2.9 Different molecular markers

There are various types of molecular markers, varying from isozymes to DNA based molecular marker types. DNA based markers are currently being widely used due to their effectiveness toward biotechnology, and are identified as marker of choice (Kumar et al., 2009). DNA based markers can be separated into two types; first non-PCR-based (RFLPs) and second; PCR-based markers, RAPDs, AFLPs, SSRs, and SNPs. No marker is superior to all others for a wide range of applications. However, the progression of molecular marker is primarily motivated by the throughput and cost of detection method and the level of reproducibility (Bernardo, 2008 cited by Mammadov et al., 2012). The latest formed SNP marker is currently the preferred marker among other DNA based markers. When searching for articles on Google Scholar, the combination of three key phrases (marker-assisted-selection, SNP, and plant breeding) showed only 637 articles for the period 1985-2005; however similar search showed approximately 4560 articles for the period 2006-2012 (Mammadov et al., 2012). Despite that, according to Roychowdhury et al. (2014) no molecular markers are available yet that fulfill all requirements needed by researchers. However, SNP markers are desirable since they meet most criteria of good markers as described in Table 2.2.

Table 2.2: Characteristics of a suitable molecular marker

| Characteristics |
| --- |
| • Polymorphic: ability to measure naturally occurring DNA polymorphism<br>• Easy and cheap to detect<br>• Easy and fast assay<br>• Co-dominant: allows evaluation of heterozygosity in allogamous populations.<br>• Easy availability<br>• Highly reproducibility<br>• Sequencing neutral DNA irrespective of the environmental conditions where the plant(s) were grown.<br>• Randomly and frequently distributed throughout the genome. |

Source: Kumar et al. (2009)

### 2.9.1 Single nucleotide polymorphisms (SNPs)

A SNP is an individual nucleotide base difference between two DNA sequences (Xu, 2010). SNPs are currently one of the most popular markers for fine mapping of heritable traits (Chagné et al., 2007). Syvänen (2001), as cited by Hayward et al. (2012) reported that the low mutation rate of SNPs makes them valued for understanding complex genetic traits and genome evolution. SNPs are the newest and highly automated genotyping techniques (Hu et al., 2012). About 100 SNPs are required to detect accurate parentage of changes in nucleotide sequences by one base substitution of natural populations (Liao and Lee, 2010). However, through SNAPshot Multiplex Assay one individual can generate over 10 000 data points per day. SNAPshot is thereby suitable for marker assisted selection of several traits simultaneously. In addition the ability to detect SNPs without the use of gels; and analysis that is suitable to high throughput and automation has led to their fondness by the breeders. This has led to markers based on SNP to quickly expand on the discipline of molecular genetics during recent years (Mammadov et al., 2012).

Many different markers have and are still being utilized to assist with maize improvement. However, soon after discovering SSRs markers in the genome of plants, they were declared as marker of choice compared to previously discovered markers. Yet, discovery of SNPs was another breakthrough because, according to Mammadov et al. (2012) "although SNPs are less polymorphic than SSR markers because of their bi-allelic nature, they easily compensate this disadvantage by being abundant, ubiquitous, and amenable to high- and ultra-high-throughput automation". Due to the abundance of SNP-based markers it is possible to generate very dense genetic maps which can be used to conduct MAS breeding programmes (Barbazuk et al., 2007). For example, maize has 1 SNP per 60–120 bp. The use of SNP markers in maize breeding is a sensible notion because, unlike polyploidy crops, such as potatoes, cotton, canola, and tobacco; the use of bi-allelic SNP marker for maize does not pose any challenges (Šimić et al., 2009).

An example where SNP markers were used successfully in maize is reported by Buckler et al. (2009), where SNP markers simplified the examination of complex traits (flowering time in maize). The authors discovered that the genetics that govern flowering time in maize is controlled by small additive quantitative trait loci (QTL) rather than a single large-effect QTL. Buckler et al. (2009), conducted a study using SNP markers for mapping resistance to northern leaf blight disease affecting maize, and 29 QTL were discovered and candidate genes were identified. Despite the challenges posed when using SNP markers to polyploidy crops, SNP markers have been successful in wheat. In one of the recent study by Lagudah et al. (2009), SNP markers were developed for locus that governs for resistance to powdery mildew, stripe rust, and leaf rust diseases. Nonetheless, since private sector does not normally release details of its breeding methodology it is believed that private SNP markers are developed by companies and are being largely used in their private breeding programmes (Ganal et al., 2009). However, millions of SNP markers have been discovered and released in maize (Mammadov et al., 2012).

## 2.11 Genotype by environment interaction

Genotype by environment interaction (GE) refers to the differential response of varieties grown in different environments (Finlay and Wilkinson, 1963). In environmental conditions influenced by drought, pest and disease pressure, low soil-fertility, and agronomical management practices results into major implications. The GE reduces response to selection for abroad adaptation in breeding (Babic et al., 2011). Through multi-location and multi-seasonal testing GE can be quantified. Statistically effective methods such as biplots based on principal component analysis have been developed for GE analysis (Crossa et al., 2002). A biplot offers a graphical display that summarizes the data of GE main effects and the principal component of scores of the interaction between genotypes and environments (Gauch, 2013). Methods such as the additive main effect and multiplicative interaction (AMMI) and the genotype main effect plus the GE (GGE) biplot have been widely used. Studies indicates that AMMI and GGE yet remain unclear in terms of differences in their effectiveness (Gauch et al. 2008), however, in the current study the AMMI approach, cultivar superiority and rank analysis methods were used.

### 2.11.1 Additive main effect and multiplicative interaction

The AMMI model comprises genotype main effect, environment main effect and the interaction with 0-F interaction's PCA axes (IPCA) (Crossa, 1990). The AMMI model is used to clarify GE and to improve accuracy of yield estimates and used for better understanding of genotypes, environments and the complex of their interactions which essentially aid in assigning genotypes to environments they are adapted to and in identifying the best environment for evaluation of genotypes (Babić et al., 2011). Crossa et al. (1990) indicated that the AMMI model can be used to analyze the GE, identify superior maize hybrid, and select for the maize hybrid in the specific test environment. Depending on the number of principal components used in the study, the AMMI models can range from AMMI (1) to AMMI (n). In the current study the AMMI (2) model was adopted.

### 2.11.2 Cultivar Superiority and rank analysis

The stability of cultivars can be defined as one with a performance near the maximum in various environments. Furthermore, potential superior cultivars are tested against successful cultivars on the market. The stability of genotypes can be studied by using methods such as superiority and rank methods. Cultivar superiority method characterizes genotypes with a parameter ($P_i$) by associating stability with productivity to identify cultivars that are both stable and high yielding (Lin and Binns, 1988). Ranking method provides information on generally good performers of the genotype at various environments (Makanda, 2009). Thus, cultivar superiority has been preferred because it also provides information on the general and specific adaptability of a genotype.

## 2.12 Conclusion and Summary of the literature review

In SSA maize has been found to be one of the most important staple crops. However, due to climatic changes and population growth the demand for maize is expected to increase. Furthermore, poor environmental conditions such drought occurrences, temperature increase pose a threat to maize productivity in addition to the use of poor improved varieties. The consumption of maize in SSA is very high, and preference of white maize over orange maize has been identified as one of the issues resulting in VAD. Problem of VAD results in night blindness, loss of appetite, poor growth rate and weakened immune system. Thus, interventions to supplement PVA in the diet are being implemented. Consumption of maize high in quality protein maize and/or high in provitamin A carotenoids has been identified as a promising strategy to alleviate PVA. HarvestPlus have based their research on crops such as orange maize and orange fleshed sweet potato as they are a major part of SSA diet compared to other source of PVA such as carrot.

It is necessary to alleviate maize productivity and nutrition through research such as improving of SSA maize varieties. Additionally, that can be attained through incorporating conventional breeding with the most recent molecular breeding. Potential of molecular markers to be used as aids in the breeding of crops has been put into practice by many researchers and it has been a

successful approach. Importantly, they used to estimate genetic distance, which aids with the assigning of inbreds into heterotic groups in order to utilize the advantages of heterosis and predict high performing hybrids. However, in many cases, the estimates of correlation between genetic distance and heterosis were statistically significant but too low to be useful for prediction. This is mainly because it cannot be guaranteed that inbred lines from different heterotic groups will always give rise to high performing hybrids. This calls for a need to further research on the relationship between genetic diversity and performance of hybrids derived from PVA and QPM inbred lines.

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# CHAPTER THREE

# CHARACTERIZATION OF TWENTY MAIZE INBRED LINES USING SINGLE NUCLEOTIDE POLYMORPHISM MARKERS


## Abstract

Knowledge of genetic diversity is very important for devising a viable breeding programme. The objectives of this study were to analyze the genetic diversity present among the Pro-Vitamin A, Quality Protein Maize and Normal Maize lines; and to identify potential heterotic groups. Twenty maize inbred lines were used in the study; ten inbred lines were in group Pro-Vitamin A were developed at UKZN; three inbred lines in group Pro-Vitamin A were developed at CIMMYT; four inbred lines in group quality protein maize were developed at Quality Seeds; and three inbred lines in group normal maize with inbred lines developed at UKZN. The 20 maize inbred lines were genotyped using 93 SNPs on the MassARRAY platform of Sequenom at the DNA Landmarks Laboratory in Canada. Data was analyzed using the PowerMarker version 3.25 statistical package. Out of 93 SNP markers used, six of them were monomorphic while 87 were polymorphic. The use of SNP markers was effective; the data set reflected the homogenously homozygous state of inbred lines and was able to determine the genetic diversity and distance. Inbred lines that showed the highest genetic distance were normal maize (DPVA17) and pro-Vitamin A (DPVA12) which was 0.54; and lowest genetic distance was observed between normal maize (DPVA19) and normal maize (DPVA18) which was 0.11. In the current study, the higher genetic diversity was observed between previously identified groups and six potential heterotic groups were identified.

## 3.1 Introduction

The assessment of genetic diversity in maize has been found to be helpful in various studies. This is because selection of genotypes depends on availability of genetic variation in the breeding material (Cholastova et al., 2011). Various markers have been used to assess genetic diversity; however Ibitoye and Akin-Idow (2010) recommended SNP markers as the leading effective markers followed by simple sequence repeat markers for studying genetic diversity and genetic distance because they are highly polymorphic, cheap and evenly distributed in the genome. Genetic diversity is essential for line improvement in maize breeding and the growing of open pollinated varieties by farmers is believed to be helpful to ensure genetic diversity for future maize breeding programmes.

Nowadays maize breeding programmes develop inbred lines and cross them to produce hybrids in order to utilize the hybrid vigour. This hybrid maize breeding was initiated in 1909 through the original research of Dr. G.H. Shull. Hybrid maize planting improves farmer's productivity and warrant a dependable, sustainable food supply because of hybrid vigour. However, hybrid vigour can only be utilized in $F_1$ generation. Inbreeding depression is observed on successive generations of self-fertilization. Utilization of heterosis as an approach for crop improvement depends on identifying best complimentary inbred lines to cross, and that can be achieved through identifying heterotic groups (Akinwale et al., 2014). Inbred lines which are genetically dissimilar have high potential of expressing high heterosis. Heterotic grouping has been achieved by calculating the genetic distance among the populations or lines.

Genetic distance can be determined by use of molecular markers and can be used to classify inbred lines into heterotic groups (Legesse et al., 2008). Genetic distances have been used in many studies to group similar lines and to identify heterotic patterns. In the past, the genetic distance analysis of maize inbred lines was based on morphological traits. Recent studies address genetic distance (GD) analysis through molecular markers (Abed and Abed, 2013). Identifying heterotic patterns through phenotype is very expensive compared to the use of molecular markers

(Semagn et al., 2012). Therefore in recent studies of heterotic patterns SNP markers have been widely used (Nelson et al., 2011).

The relationship between genetic distance and heterosis is still not well understood. Some studies noticed a complex one while others noticed a linear relationship. Reif et al. (2013) conducted a study on sunflower and observed a complex relationship, while George et al. (2011) conducted a study on maize and observed a linear relationship. Therefore, the objectives of this current study were to analyze the genetic diversity present among the Pro-Vitamin A (UKZN), Pro-Vitamin A (CIMMYT), Quality Protein Maize (Quality Seeds) and Normal Maize (UKZN) lines.

## 3.2 Methods and materials

### 3.2.1 Plant Material

Twenty maize inbred lines (Table 3.1) were used in the study. Inbred lines DPVA1-DPVA10 share characteristic of high pro-Vitamin A and were developed at University of KwaZulu Natal (UKZN). Inbred lines DPVA11, DPVA12 and DPVA13 share characteristic of high pro-Vitamin A and were developed at CIMMYT. Inbred lines DPVA14, DPVA15 and DPVA16 share characteristic of quality protein. Inbred lines DPVA17, DPVA18 and DPVA19 are normal maize lines which are high yielding and prolific while inbred line DPVA20 is a quality protein maize inbred line from the Quality Seeds.

Table 3.1: Main features of twenty maize inbred lines used in the current study

| Name | Origin | Characteristics |
|---|---|---|
| **DPVA1** | UKZN | PVA line, long ear, slight lodging, prolific |
| **DPVA2** | UKZN | PVA line, long ears, good standing ability |
| **DPVA3** | UKZN | PVA line, very prolific, good standing ability, high seed yield |
| **DPVA4** | UKZN | PVA line, long ear, good standing ability |
| **DPVA5** | UKZN | PVA line, long ear, good cob, low lodging |
| **DPVA6** | UKZN | PVA line, high seed yield, good standing ability |
| **DPVA7** | UKZN | PVA line, long cob, low lodging |
| **DPVA8** | UKZN | PVA line, high seed yield, good standing ability |
| **DPVA9** | UKZN | PVA line, very long ear, good standing ability, good yield |
| **DPVA10** | UKZN | PVA line, long ear, good standing ability |
| **DPVA11** | CIMMYT | PVA line, long cob, high yield and high vitamin A |
| **DPVA12** | CIMMYT | PVA line, long cob |
| **DPVA13** | CIMMYT | PVA line, short cob |
| **DPVA14** | Quality Seeds | QPM line, medium cob, yellow |
| **DPVA15** | Quality Seeds | QPM line, medium cob, yellow |
| **DPVA16** | Quality Seeds | QPM line, long cob, yellow |
| **DPVA17** | UKZN | High yield, prolific, yellow normal maize |
| **DPVA18** | UKZN | High yield, prolific, yellow normal maize |
| **DPVA19** | UKZN | High yield, prolific, yellow normal maize |
| **DPVA20** | Quality Seeds | QPM line, temperate, high yield potential, yellow |

PVA= Pro-Vitamin A, QPM= Quality Protein Maize, CIMMYT= International Maize and Wheat Improvement Center, and UKZN= University of KwaZulu Natal.

### 3.2.2 Plant material preparation and DNA extraction

Inbred lines were planted in pots, where two seeds per pot were planted and replicated four times per inbred line at the UKZN tunnel. Drip fertigation system was used, where each pot had one dripping head. In accordance with the protocol supplied by the DNA Landmarks Laboratory, two leaf discs from two plants per inbred line were harvested at the four leaf stage, and put into specific well position of a block. The block was than sealed with Pore Tape and placed inside a plastic bag. About 50 g of silica gel (desiccant) was put inside the plastic bag to control humidity and avoid degradation of the leaf discs harvested. The samples were then sent to the DNA landmarks laboratory in Canada for genotyping. DNA extraction was done using a Sarkosyl based method (Hasan et al., 2008) at the DNA Landmarks laboratory in Canada. The leaf material was ground into a fine powder in liquid nitrogen after which 3 ml of DNA extraction buffer (100 mM Tris-HCl at pH 8.0, containing 0.35 M sorbitol, 5 mM EDTA at pH 8.0, and 1% 2-mercaptoethanol) and 1 ml of phenol was added in a test tube and homogenised. Another 2 ml of phenol was added and centrifuged at 12 000 rpm for 5 min. The supernatant was transferred into an equal volume of 200 µl of ice-cold 95% ethanol and centrifuged at 12 000 rpm for 5 min to precipitate the DNA. The precipitated DNA was washed in 70% ethanol, dissolved in 0.5 ml of Tris EDTA (TE) with 2 µg of RNAase and incubated at 37$^{o}$C for 30 min. Then further 0.25 ml phenol and 0.25 ml chloroform was added and centrifuged and the upper phase was transferred into a fresh tube with an equal volume of 95% ethanol for DNA precipitation. The precipitated DNA was again washed with ice-cold 70% ethanol and dissolved in 0.2 ml of TE.

### 3.2.3 SNP selection and amplification

The maize leaf samples of the 20 maize inbred lines were genotyped using 93 SNP markers on the MassARRAY platform of Sequenom at the BASF Plant Science Centre, DNA Landmarks (84 Rue Richelieu, Quebec, Canada). The genotyping was done following the proprietary standard protocols of DNA Landmarks Inc. Polymerase chain reaction (PCR) mixes were prepared for each sample containing 5x PCR Buffer, 2.5 mM dNTPs, 25 mM $MgCl_2$, 10 mM of each primer, 5 µ of DNA polymerase and 25 ng $\mu l^{-1}$ of genomic DNA. Cycling parameters were as follows: 94°C for 5 min, followed by 45 cycles of 94°C for 20 s, annealing step for 30 s at 56°C, extension step at 72°C for 1 min and a polishing step at 72°C for 3 min (Gabriel et al.,

2009). The DNA quality was evaluated carefully before genotyping by screening each sample on a 0.8% (w/v) agarose gel. Once the DNA quality passed the quality control, the DNA samples were used for SNP genotyping by a commercially available Sequenom MassARRAY platform following the standard protocols described by Gabriel et al. (2009). The protocol for this assay recommended using 2.5 ng $\mu l^{-1}$ DNA per sample. Out of the 93 SNPs markers used, six were monomorphic while 87 were polymorphic. Therefore only the 87 were used for data analysis.

### 3.2.4 Statistical analysis

The SNP data was analyzed using the PowerMarker version 3.25 statistical package (Liu et al., 2003) to determine parameters such as availability of markers, gene diversity, observed heterozygosity, expected heterozygosity, polymorphic information content (PIC), inbreeding coefficient, and major allele frequency as described by Boistein et al. (1980). The analysis included estimation of genetic distance, and construction of the dendrogram based on unweighted paired group method using arithmetic averages (UPGMA) interpreting the genetic relatedness of 20 inbred lines.

Expected heterozygosity (He), which is the probability that two alleles from the same locus would be different when chosen at random, was calculated for each SNP locus according to Nei (1973) as; $He = 1 - \Sigma (p_i)^2$. Observed heterozygosity ($H_o$), was calculated by dividing the number of heterozygous individuals by the number of individuals scored. The expected heterozygosity and observed heterozygosity were used to evaluate the genetic diversity within the set of inbred lines. Polymorphic information content (PIC) for the SNP markers in the sample DNA was calculated according to Boistein et al. 1980 as $1- \Sigma p_i^2$ where $\Sigma$ stands for summation over all alleles; $p_i$ is the frequency of the $i^{th}$ allele in a locus for individual p.

## 3.3 Results

### 3.3.1 Genetic diversity, distance, and similarity assessment among inbred lines

Total of 93 SNP markers were used to genotype 20 maize inbred lines, however among the 93 only 87 were found to be polymorphic while the remaining 6 were monomorphic. The results in Table 3.2 shows that the PIC value of the polymorphic SNP markers, which is a measure of allele diversity at a locus, to range from 0.091 to 0.375 with an average of 0.287. Expected heterozygosity results showed a range of 0.0 to 0.1 with a mean of 0.00517, where most of the markers were showing 100% level of homozygosity this can also be verified by the mean of inbreeding coefficient which was observed to be 0.986.

The results in Table 3.3, show genetic distances for each pair among the twenty inbred lines. It can be observed from the results that the highest genetic distance (GD) was between normal maize (DPVA17) and Pro-Vitamin A (DPVA12) which was 0.54; and the lowest GD was between normal maize (DPVA19) and normal maize (DPVA18) which was 0.11. The results also showed relatively lower genetic distances among inbred lines DPVA01 to DPVA10 which share the same origin, UKZN and are Pro-Vitamin A lines. GDs between inbred lines of group PVA (CIMMYT) ranged from 0.15 to 0.44; GDs between inbred lines of group quality protein maize (Quality Seeds) ranged from 0.35 to 0.49; and GDs between inbred lines of group normal maize (UKZN) ranged from 0.11 to 0.22 which was fairly low. DPVA20 showed higher GD range of 0.37 to 0.52 when paired to all other inbred lines.

The similarity percentage indices shown in Table 3.4 show a linear relationship between GD and similarity, where the normal maize (DPVA17) and pro-Vitamin A (DPVA12) had the highest GD in Table 3.3 and has the lowest similarity in Table 3.4; and normal maize (DPVA19) and normal maize (DPVA18) had the lowest GD in Table 3.3 and has the highest similarity in Table 3.4.

The dendrogram based on UPGMA shown in Figure 3.1 has six clusters at 0.18 level of dis-similarity, most of the clusters were consistence with the origin and major identified characteristic of the inbred lines. The largest cluster which is cluster 3, had 9 out of 10 inbred lines from the PVA (UZKN) group the odd line was DPVA13 which is PVA line from CIMMYT; cluster 2 had 2 out of 3 inbred lines from PVA (CIMMYT) group; cluster 6 had 2 out of 3 inbred lines from QPM (Quality Seeds) group; cluster 5 had 3 inbred lines from NM (UKZN) group; cluster 4 had one inbred line from QPM (USA) group; and cluster 1 had DPVA16 which belonged to QPM (Quality Seeds) group.

Table 3.2: Average and range of polymorphism for 20 lines assayed with 87 SNP markers.

| **Parameter** | **Average** | **Range** |
|---|---|---|
| Availability | 0.995 | 0.7-1.0 |
| Gene diversity | 0.360 | 0.095-0.5 |
| Heterozygosity | 0.005 | 0.0-0.1 |
| PIC | 0.287 | 0.091-0.375 |
| Inbreeding coefficient | 0.986 | 0.773-1.0 |
| Major allele frequency | 0.721 | 0.500- 0.950 |

Table 3.3: Genetic distances for each pair among the 20 maize inbred lines based on 87 SNP markers

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **DPVA1** | 0.00 | | | | | | | | | | | | | | | | | | | |
| **DPVA2** | 0.17 | 0.00 | | | | | | | | | | | | | | | | | | |
| **DPVA3** | 0.35 | 0.28 | 0.00 | | | | | | | | | | | | | | | | | |
| **DPVA4** | 0.39 | 0.32 | 0.36 | 0.00 | | | | | | | | | | | | | | | | |
| **DPVA5** | 0.31 | 0.21 | 0.32 | 0.43 | 0.00 | | | | | | | | | | | | | | | |
| **DPVA6** | 0.30 | 0.21 | 0.36 | 0.25 | 0.24 | 0.00 | | | | | | | | | | | | | | |
| **DPVA7** | 0.31 | 0.26 | 0.25 | 0.37 | 0.18 | 0.28 | 0.00 | | | | | | | | | | | | | |
| **DPVA8** | 0.38 | 0.28 | 0.29 | 0.41 | 0.32 | 0.35 | 0.26 | 0.00 | | | | | | | | | | | | |
| **DPVA9** | 0.40 | 0.27 | 0.23 | 0.34 | 0.28 | 0.27 | 0.26 | 0.26 | 0.00 | | | | | | | | | | | |
| **DPVA10** | 0.28 | 0.23 | 0.33 | 0.29 | 0.27 | 0.18 | 0.31 | 0.38 | 0.34 | 0.00 | | | | | | | | | | |
| **DPVA11** | 0.39 | 0.31 | 0.40 | 0.43 | 0.37 | 0.31 | 0.37 | 0.39 | 0.33 | 0.36 | 0.00 | | | | | | | | | |
| **DPVA12** | 0.48 | 0.40 | 0.45 | 0.47 | 0.46 | 0.40 | 0.41 | 0.44 | 0.35 | 0.40 | 0.15 | 0.00 | | | | | | | | |
| **DPVA13** | 0.24 | 0.21 | 0.36 | 0.45 | 0.29 | 0.26 | 0.29 | 0.34 | 0.39 | 0.31 | 0.35 | 0.44 | 0.00 | | | | | | | |
| **DPVA14** | 0.38 | 0.34 | 0.42 | 0.29 | 0.45 | 0.38 | 0.41 | 0.45 | 0.39 | 0.38 | 0.35 | 0.40 | 0.35 | 0.00 | | | | | | |
| **DPVA15** | 0.36 | 0.38 | 0.44 | 0.37 | 0.53 | 0.45 | 0.47 | 0.50 | 0.45 | 0.41 | 0.45 | 0.45 | 0.44 | 0.35 | 0.00 | | | | | |
| **DPVA16** | 0.43 | 0.38 | 0.46 | 0.38 | 0.42 | 0.41 | 0.45 | 0.44 | 0.46 | 0.51 | 0.42 | 0.49 | 0.49 | 0.44 | 0.49 | 0.00 | | | | |
| **DPVA17** | 0.36 | 0.40 | 0.42 | 0.35 | 0.39 | 0.39 | 0.39 | 0.48 | 0.41 | 0.39 | 0.50 | 0.54 | 0.38 | 0.33 | 0.36 | 0.51 | 0.00 | | | |
| **DPVA18** | 0.41 | 0.44 | 0.40 | 0.38 | 0.40 | 0.44 | 0.38 | 0.47 | 0.36 | 0.46 | 0.41 | 0.50 | 0.45 | 0.38 | 0.36 | 0.49 | 0.22 | 0.00 | | |
| **DPVA19** | 0.38 | 0.42 | 0.42 | 0.38 | 0.42 | 0.46 | 0.36 | 0.46 | 0.38 | 0.48 | 0.43 | 0.49 | 0.43 | 0.40 | 0.41 | 0.48 | 0.16 | 0.11 | 0.00 | |
| **DPVA20** | 0.47 | 0.41 | 0.41 | 0.36 | 0.52 | 0.39 | 0.45 | 0.49 | 0.47 | 0.44 | 0.44 | 0.40 | 0.45 | 0.45 | 0.38 | 0.44 | 0.41 | 0.39 | 0.37 | 0.00 |

1=DPVA1; 2=DPVA2; 3=DPVA3;…DPVA20

Table 3.4: Similarity matrix for the 20 maize inbred lines based on 87 SNP markers.

| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **DPVA1** | 1.00 | | | | | | | | | | | | | | | | | | | |
| **DPVA2** | 0.72 | 1.00 | | | | | | | | | | | | | | | | | | |
| **DPVA3** | 0.50 | 0.58 | 1.00 | | | | | | | | | | | | | | | | | |
| **DPVA4** | 0.47 | 0.54 | 0.50 | 1.00 | | | | | | | | | | | | | | | | |
| **DPVA5** | 0.55 | 0.67 | 0.54 | 0.43 | 1.00 | | | | | | | | | | | | | | | |
| **DPVA6** | 0.56 | 0.67 | 0.49 | 0.63 | 0.63 | 1.00 | | | | | | | | | | | | | | |
| **DPVA7** | 0.55 | 0.61 | 0.62 | 0.49 | 0.71 | 0.58 | 1.00 | | | | | | | | | | | | | |
| **DPVA8** | 0.48 | 0.58 | 0.58 | 0.44 | 0.54 | 0.50 | 0.61 | 1.00 | | | | | | | | | | | | |
| **DPVA9** | 0.46 | 0.60 | 0.65 | 0.51 | 0.58 | 0.60 | 0.60 | 0.61 | 1.00 | | | | | | | | | | | |
| **DPVA10** | 0.59 | 0.64 | 0.53 | 0.57 | 0.60 | 0.71 | 0.55 | 0.48 | 0.52 | 1.00 | | | | | | | | | | |
| **DPVA11** | 0.47 | 0.54 | 0.45 | 0.43 | 0.48 | 0.55 | 0.49 | 0.46 | 0.53 | 0.50 | 1.00 | | | | | | | | | |
| **DPVA12** | 0.38 | 0.45 | 0.41 | 0.39 | 0.40 | 0.45 | 0.45 | 0.42 | 0.51 | 0.45 | 0.75 | 1.00 | | | | | | | | |
| **DPVA13** | 0.63 | 0.67 | 0.49 | 0.41 | 0.57 | 0.60 | 0.57 | 0.52 | 0.46 | 0.55 | 0.51 | 0.42 | 1.00 | | | | | | | |
| **DPVA14** | 0.47 | 0.52 | 0.43 | 0.57 | 0.41 | 0.47 | 0.45 | 0.41 | 0.47 | 0.47 | 0.50 | 0.46 | 0.51 | 1.00 | | | | | | |
| **DPVA15** | 0.49 | 0.47 | 0.42 | 0.49 | 0.34 | 0.40 | 0.39 | 0.36 | 0.41 | 0.45 | 0.41 | 0.40 | 0.42 | 0.50 | 1.00 | | | | | |
| **DPVA16** | 0.43 | 0.48 | 0.40 | 0.48 | 0.44 | 0.44 | 0.41 | 0.42 | 0.40 | 0.36 | 0.44 | 0.37 | 0.37 | 0.42 | 0.37 | 1.00 | | | | |
| **DPVA17** | 0.50 | 0.45 | 0.43 | 0.50 | 0.46 | 0.46 | 0.47 | 0.38 | 0.45 | 0.46 | 0.36 | 0.33 | 0.48 | 0.53 | 0.50 | 0.36 | 1.00 | | | |
| **DPVA18** | 0.45 | 0.42 | 0.45 | 0.48 | 0.46 | 0.42 | 0.48 | 0.39 | 0.49 | 0.40 | 0.45 | 0.36 | 0.41 | 0.48 | 0.49 | 0.38 | 0.65 | 1.00 | | |
| **DPVA19** | 0.47 | 0.43 | 0.43 | 0.48 | 0.44 | 0.40 | 0.49 | 0.40 | 0.47 | 0.38 | 0.43 | 0.37 | 0.43 | 0.46 | 0.45 | 0.38 | 0.74 | 0.82 | 1.00 | |
| **DPVA20** | 0.39 | 0.44 | 0.45 | 0.49 | 0.35 | 0.46 | 0.41 | 0.37 | 0.39 | 0.42 | 0.42 | 0.45 | 0.41 | 0.40 | 0.47 | 0.42 | 0.44 | 0.46 | 0.49 | 1.00 |

1=DPVA1; 2=DPVA2; 3=DPVA3;…DPVA20

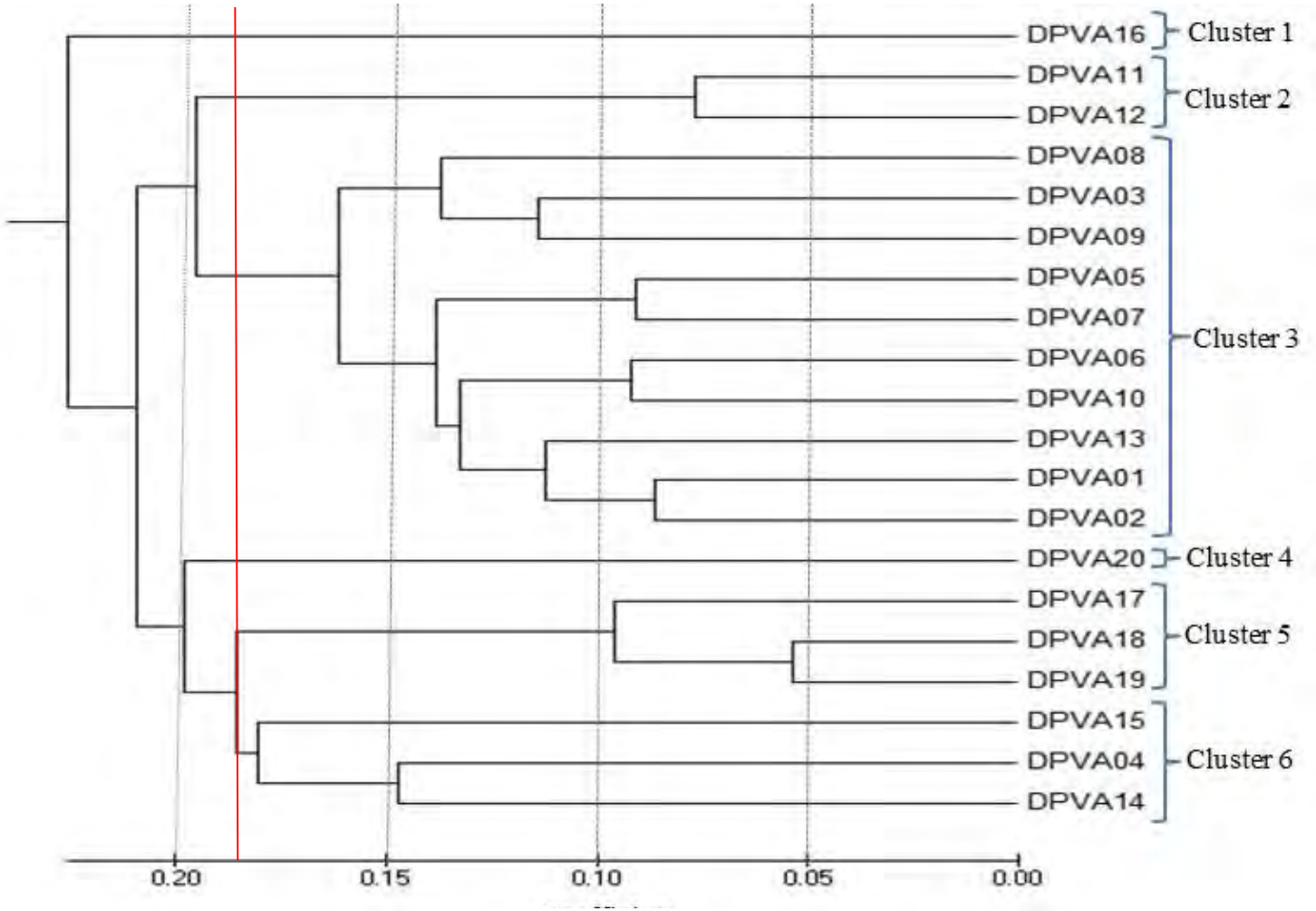


Figure 3.1: Dendrogram based on UPGMA interpreting the genetic relatedness of 20 inbred lines based on 87 SNPs markers

### 3.4 Discussion

In this study, SNP markers were successfully used to genotype maize inbred lines. Genetic diversity can be expressed by the number of alleles observed within the analyzed populations, individuals, or lines. In the current study the average number of alleles was 0.287. Legesse et al. (2007) conducted a study to investigate the level of genetic diversity among maize inbred lines and assess their genetic structures by applying simple sequence repeat (SSR) markers. They identified average polymorphism information content (PIC) of 0.58 and genetic distance expressed as Euclidean distance, varying from 0.28 to 0.73 with an average of 0.59. Legesse et al. (2007) concluded that the genetic diversity observed is large enough for maximized heterosis. The average number of alleles observed in the current study is slightly lower than the number that has been reported in the literature, especially when SSR markers were used. This is because of the use of SNP markers which are usually known to be biallelic (Vignal et al., 2002). SNP markers also verified the successful self-fertilization of inbred lines used in the study with minimal contamination because a 0.986 inbreeding coefficient was observed, which reflects the homogenously homozygous state of the inbred lines used in the study. Van Inghelandt et al. (2010) conducted a study to examine genetic diversity and population structure in elite maize germplasm based on SSR and SNP markers. A total 1 537 elite maize inbred lines were genotyped with 359 SSR and 8 244 SNP markers, and a gene diversity of 0.32 based on SNP markers was identified which is a considerably lower estimate and also inaccordance with the current study which showed a genetic diversity of 0.36.

The majority of the genetic distances were above 0.3 which is advantageous because it indicated that most of the inbred lines are dissimilar, thereby chances of getting desirable $F_1$ hybrids are high. Several studies have shown that for the production of hybrids with better yield performance, it might be best to use inbred lines with larger genetic distances to maximize hybrid vigour (Biswas et al., 2008). In Table 2.3, inbred lines that showed the highest genetic distance were, normal maize (DPVA17) and pro-Vitamin A (DPVA12) which was 0.54 and this indicates that these two inbred lines are the most genetically diverse. The main reason behind, is that they are from different groups; DPVA17 is high yielding, prolific, normal maize (without

both PVA and QPM) developed at UKZN, and DPVA12 is high in pro-Vitamin A, has long cob, and was developed at CIMMYT using different germplasm.

Lowest genetic distance was observed between normal maize (DPVA19) and normal maize (DPVA18) which was 0.11. This serves as a confirmation that inbred lines from a similar group are more likely to be similar compared to between the groups. The lines DPVA19 and DPVA18 inbred lines are both found in the normal maize group of inbred lines developed at UKZN from the same genetic population. For most inbred lines that share the same group the genetic distances observed were fairly low, and conversely for inbred lines that are at different groups the genetic distances observed were fairly high. The magnitude of the genetic distance between any two parents in the current study was indirectly proportional to shared origin and/or characteristic. Inbred lines with low genetic distances and higher similarity index results into minimal hybrid vigour when crossed, therefore superior $F_1$ hybrids can be derived when crossing inbred lines with the highest genetic distance.

In the current study the PIC mean was 0.287 which is a lower value than a value close 0.33 reported by Legesse et al. (2007). Six potential cluster groups were identified. The dendrogram based on UPGMA (Figure 2.1) shows that SNP markers were successfully used to genotype maize inbred lines, because most clusters have almost all inbred lines from the same group. Cluster 1, shows DPVA16 to be very distinct from all 19 inbred lines, and this is also confirmed by fairly higher GD of DPVA16 when pairing with other inbred lines; however DPVA16 was expected to be on the similar cluster with DPVA14 and DPVA15. Nine out of ten inbred lines from the group of Pro-Vitamin A developed at UKZN were observed in the similar cluster, DPVA4 was the only inbred line in the group to be placed in a different cluster group. DPVA4 was placed with DPVA14 and DPVA15 which both belong to quality protein maize developed at Quality Seeds. DPVA20 is a QPM inbred line like DPVA14, DPVA15, and DPVA16. However, DPVA20 was grouped in a separate group because of its origin and hence separated from the other QPM inbred lines which is USA. Inbred lines DPVA16 and DPVA20 are potential

candidates to use when maximizing heterosis. Furthermore, all being QPM they have a chance to be combined with PVA lines

### 2.5. Conclusions

Single nucleotide polymorphism markers were effective because most of the inbred lines that have similar pedigree and origin were clustered together. Inbred lines were clustered according to the existing groups based on pedigree data of the lines which confirm that accurate heterotic grouping may be achieved when SNPs are used for genotyping. Fairly higher genetic distances were observed in most inbred lines this implies that there is huge diversity and a high potential for producing superior hybrids. In the current study, six potential heterotic groups were identified. However this will be confirmed by conducting trials of hybrids between these lines.

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# CHAPTER FOUR

## SELECTION OF SUPERIOR HYBRIDS AT FOUR DIFFERENT SITES AND EVALUATION OF ASSOCIATION OF TRAITS TO YIELD


### Abstract

Progressive maize breeding programmes select hybrids using data from multi-location studies to account for genotype x environment interaction (GE) and to identify the most stable hybrids. The objectives of the study were to identify top 10% hybrids in terms of hybrid stability and to identify traits associated with high yield potential across four environments in KwaZulu-Natal in hybrids developed from 4 QPM testers and 10 PVA new experimental lines. A 4 x 10 North Carolina design II with reciprocals mating scheme was conducted to generate 80 experimental hybrids with adequate seed for planting in trials but only 78 experimental hybrids were successful. Three check hybrids, DKC80-40BRGEN, 11C1774, and 11C1483 were used in the study. The 81 hybrids were evaluated in a 9 × 9 alpha-lattice design with two replications at four sites. The data collected was analyzed using Genstat and SAS softwares. Grain yield was highly significant ($p<0.001$) at Cedara and Dundee, and not significant at Jozini and Ukulinga. The number of ears per plant, grain moisture content and ear aspect were highly significant ($p<0.001$) at Cedara and Dundee, and significant ($p<0.05$) at Jozini and Ukulinga. Genotype x environment interaction was observed, because performance of genotypes varied with sites. At the Cedara Research Station, the highest performing experimental hybrid was 14PVAH-29; 14PVAH-166 at Dundee, 14PVAH-8 at Jozini, and 14PVAH-50 at Ukulinga, which is consistent with observation of GE in the overall analysis. From the three different methods used to identify the hybrid stability variation was observed in term of the results each method yielded. However experimental hybrids 14PVA-139, 14PVA-129 and 14PVA-10 showed highest stability among the three methods. The current study showed that traits such as number of ears per plant, plant height, and ear aspect were strongly and positively associated with grain yield potential of the hybrids, and that stem lodging, root lodging, and ear rot were negatively associated with yield.

## 4.1 Introduction

Hybrid maize planting improves farmer's productivity and warrant a dependable, sustainable food supply because of hybrid vigour. This hybrid vigour results from crossing two genetically unrelated inbred parents to create a hybrid. There are two distinguished steps in hybrid breeding, one is to develop inbred lines, and the other is to select proper parent inbred lines to combine to give best hybrids (Lee and Tollenaar, 2007). Therefore, for step two the genetic information of both parents is very important. Pedigree and origin of inbred lines have been used successfully in several studies to predict parental lines with a promising combining ability. Combining ability is essential for hybrid breeding. However, the genetic basis of combining ability remains unclear and has been seldom investigated (Qi et al., 2013).

The main trait used when selecting for a best hybrid is yield. However yield is a dependent to many secondary traits that affect it directly and/or indirectly. Tollenaar and Lee (2011), suggested the genetic improvement of traits that are associated with yield as a strategy to improve yield potential. Therefore, for a selected hybrid other vital factors and traits such as disease resistance, grain moisture content, nutrition composition, stability and adaptability should be taken into consideration. Talking one of the considered trait as an example; grain moisture content affects the mass of grain, market value, as well as grain storability, thus moisture content may affect profit (Hellevang, 2011). Recommended grain moisture content differs with country and the usage of grain. In South Africa, it is currently set at 12.5%. Therefore, a breeder may breed for earliness to allow adequate physiological drying before the season ends.

The best hybrid cannot be selected from one environmental site due genotype x environment interaction (GE), therefore different environments must be used for hybrid selection. Engelsing et al. (2012), recommended the use of both the environmental adaptability and the stability of maize grain yield across various environments when selecting the best genotype. This is because the main weakness in the selection of genotypes with high yield capacity in different environments is the GE (Mendes et al., 2012). Numerous methods of adaptability and stability

analysis have been suggested, where the most preferred are the simplest and those that incorporate adaptability and stability as well as yield in a single statistic such as the methods of Lin and Binns (1988), Annicchiarico (1992) and Cruz et al. (2004) as cited by Mendes et al. (2012).

The breeder's main objective is to acquire genetic gain after selecting the best performing hybrids to be brought forward for further selection. This is because studies have shown that with every genetic breeding programme they can be either an increase or a reduction in the yield potential (de Toledo et al., (1990) cited by Lange and Federizzi., 2009).

The objectives of the study were as follows:

- To identify the top 10% hybrids in terms of hybrid stability across four sites based on yield and economic traits.
- To identify traits associated with yield across four environments.

## 4.2 Methods and Materials

### 4.2.1 Germplasm

Among the twenty inbred lines listed in chapter two fourteen were used in the present study (four QMP lines and ten PVA lines); a 4 x 10 North Carolina design II with reciprocals was conducted to generate 80 $F_1$ single cross hybrids. However, experimental hybrids generated were 78 because the reciprocals of DPVA4 X DPVA20, and of DPVA8 X DPVA20 failed to produce adequate seed for planting in field trials. Standard check hybrids used were; DKC80-40BRGEN (commercial hybrid which is widely grown in South Africa), 11C1774, and 11C1483 (advanced experimental hybrids from the programme at UKZN). In the present study, a total of 81 hybrids were evaluated where three were three standard checks and 78 experimental hybrids.

### 4.2.2. Experimental design, environments and management

The experimental design used in at all sites was 9 x 9 alpha-lattice design with two replications. Hybrids were evaluated in four sites (Cedara, Dundee, Jozini, and Ukulinga), where each row plot was 5 m, in row spacing was 0.3 m while inter-row spacing was 0.9 m. All sites were planted during summer season of 2013/14, and two boarder rows were planted at the ends, around the experimental sites.

Table 4.1: Geographical co-ordinates of four experimental sites

| Sites | Latitude | Longitude | Altitude (meters above sea level) |
|---|---|---|---|
| **Cedara** | 29°.54'S | 30°.26'E | 1066 |
| **Dundee** | 28°.13'S | 30°.31'E | 1217 |
| **Jozini** | 27°.39'S | 32°.10'E | 77 |
| **Ukulinga** | 29°.66'S | 30°.40'E | 808 |

A total of 250 kg/ha NPK (56N: 83P: 111K) compound fertilizer was applied as basal dressing during planting, immediately after planting curator was applied around the experimental site to repel rodents. The field was irrigated to establish the crop. Six weeks after planting, 250 kg/ha of lime ammonium nitrate (LAN 28% N) was applied as a top dressing. Weed control was achieved through both chemical such as Basagran (to kill nutsedge), Gramoxone (all green weeds) and Troopers (broadleaf weeds including morning glory) and hand weeding, and all sites were rainfed until hand harvesting after physiological maturity.

### 4.2.3. Data collection

Traits were measured following standard protocols used at CIMMYT (Magorokosho et al., 2009):

- **Plant height:** distance between the base of a plant to the insertion of the first tassel branch of the same plant.
- **Ear height:** distance between the base of a plant to the insertion of the top ear of the plant.
- **Number of plants:** number of plants harvested per plot.
- **Number of ears:** number of ears harvested per plot.
- **Field weight:** Mass of all ears harvested per plot.
- **Grain yield:** Shelled grain mass per plot adjusted to 12.5% moisture content.
- **Grain moisture content:** Percent water content on grain measured at harvest.
- **Ear aspect:** Rated on a scale from 1= very poor to 10= excellent.
- **Stem lodging:** Percentage of plants per plot that show stem lodging i.e. inclination more than 45%
- **Root lodging:** Percentage of plants per plot that show their stems inclining by more than 45%.
- **Ear rot:** Percentage of ears which are rotten.
- **Grain texture:** Rated on a scale from 1=flint to 5=dent.
- **Days-to-mid pollen:** Number of days after planting when 50% of the plants shed pollen.
- **Days-to-mid silking:** Number of days after planting when 50% of the plants show silks.
- **Anthesis-silking-interval:** Days-to-mid silking minus days-to-mid pollen.
- **Gray leaf spot:** Rated on a scale from 1= 100% leaf surface with symptoms to 9= No symptoms
- **Phaeosphaeria leaf spot:** Rated on a scale from 1= 100% leaf surface with symptoms to 9= No symptoms
- **Northern leaf blight:** Rated on a scale from 1= 100% leaf surface with symptoms to 9= No symptoms
- **Shelling percentange:** Mass of all grain shelled from the ear over mass of the whole ear

### 4.2.4. Data analysis

Data collected for all hybrids was subjected to analysis of variance, using GenStat 14th Edition and each trait was analyzed according to the following model:

$$\mathbf{Y_{ij} = \mu + \beta_i + T_j + E_{ij}}$$

Where, $Y_{ij}$ = yield of $i^{th}$genotype in the $j^{th}$ block,

$\mu$ = grand mean,

$\beta_i$ = effect of the $j^{th}$ block,

$T_j$ = effect of the $i^{th}$ genotype,

$E_{ij}$ = random error for the $i^{th}$ genotype in the $j^{th}$ block.

Genetic gains, based on the 10% (k = 1.775) selected experimental hybrids was estimated using excel, in accordance with the following models of Nyquist and Baker (1991) as cited by Wiggins (2012):

$$\mathbf{R \text{ or } \Delta G = h^2 S = h^2(\mu_2 - \mu_1)}$$

$$\mathbf{\Delta G}\ (\%) = \left(\frac{\mu_2 - \mu_1}{\mu_1}\right) * \mathbf{100}$$

Where, $\mu_2$ = population mean after selection,

$\mu_1$ = original population mean,

R= response to selection,

$\Delta G$ = genetic gain.

Stability coefficients displaying cultivar superiority index ($P_i$) and mean ranks were also computed using GenStat 14th Edition (Payne et al., 2011). Stability of the hybrids across the environments was estimated by cultivar superiority index ($P_i$) according to the following model Lin and Binns (1988):

$$\boldsymbol{P_i} = \sum_{\boldsymbol{j=1}} \boldsymbol{n}\left(\frac{\boldsymbol{X_{ij}} - \boldsymbol{M_j}}{\boldsymbol{n}}\right)^{\mathbf{2}}$$

Where, n = number of locations,

$X_{ij}$ = yield of the $i^{th}$ cultivars in the $j^{th}$ environment,

$M_j$ = maximum yield recorded in the $j^{th}$ environment.

Stability of the hybrids across the environments was also estimated by the cultivar mean rank according to the following model (Huhn, 1979):

$$\boldsymbol{S^3} = \sum_{\boldsymbol{j}}\left(\frac{\boldsymbol{r_{ij}} - \boldsymbol{r_i}}{\boldsymbol{r_i}}\right)^{\mathbf{2}}$$

Where, $S^3$ = Non-parametric statistic,

$r_{ij}$ = rank of $i^{th}$ genotype in $j^{th}$ environment,

$r_i$ = mean of ranks over all environment for $i^{th}$ genotype.

Data was subjected to AMMI using Genstat 14th Edition with the following model as described by Crossa et al. (1990):

$$\boldsymbol{Y_{ij}} = \mu + \boldsymbol{G_i} + \boldsymbol{E_j} + \sum_{\boldsymbol{k=1}}^{\boldsymbol{n}}\left(\boldsymbol{\lambda_k \alpha_{ik} Y_{jk}}\right) + \boldsymbol{e_{ij}}$$

Where, $Y_{ij}$ = is the yield of the $i^{th}$ genotype in the $j^{th}$ environment,

$\mu$ = is the grand mean,

$G_i$ = genotype deviations from the grand mean,

$E_j$ = environment deviations from the grand mean,

$\lambda_k$ = is the Eigen value of the PCA analysis axis k,

$\alpha_{ik}$ = genotype principal component scores for axis k,

$\gamma_{jk}$ = environment principal component scores for axis k,

n = number of principal components retained in the model,

$e_{ij}$ = random experimental error.

## 4.3 Results

### 4.3.1 Cedara

The results shown in Table 4.2 primarily represent the top 10% of high yielding experimental hybrids. It can be observed that selected experimental hybrids performed better than the control hybrids. However, based on LSD the significant difference was observed only on the top four experimental hybrids when compared to control hybrid 11C1483. Yield and all economic traits were highly significantly ($p<0.001$) different and the CVs for all the traits are statistically accepted for a good quality data since there are less than 15%, except for ear per plant which had a CV of 23%.

The results presented in Figure 4.1 shows distributions of hybrids, where the hybrids in quadrant labeled A are both high yielding and prolific, while hybrids on quadrant B are also high yielding. The hybrids in order are as follows, hybrid (14PVAH-29, 14PVAH-86, 14PVAH-149, and 14PVAH-107). The results presented in Figure 4.2 shows distributions of hybrids, where the hybrids in quadrant labeled A and B are most desirable due to both high yield and good ear aspect. The desirable hybrids in order are as follows, hybrid (14PVAH-86, 14PVAH-29, 14PVAH-149 and 14PVAH-107), and the least hybrid to be considered for advance would be the

one on quadrant labeled D which is hybrid 14PVAH-40. In Figure 4.3 hybrid 14PVAH-86, 14PVAH-29, 14PVAH-107 and 14PVAH-149 distributed within quadrant A was the most desirable ones due to high yield and low grain moisture content.

Table 4.2: Hybrid rank at Cedara with respect to mean grain yield and economic traits

| | Adjusted Yield (t ha$^{-1}$) | | Economic Traits | | |
|---|---|---|---|---|---|
| **Rank** | **Hybrid name** | **Mean yield** | **EPP** | **EA (1-10)** | **GMC (%)** |
| | | **Top 8 Experimental Hybrids** | | | |
| **1** | 14PVAH-29 | 10.1 | 1.97 | 6.7 | 15.5 |
| **2** | 14PVAH-86 | 9.6 | 1.66 | 8.0 | 14.8 |
| **3** | 14PVAH-149 | 9.2 | 1.88 | 7.0 | 14.2 |
| **4** | 14PVAH-107 | 9.1 | 1.88 | 7.5 | 15.4 |
| **5** | 14PVAH-129 | 8.7 | 1.46 | 8.0 | 14.6 |
| **6** | 14PVAH-70 | 8.7 | 1.64 | 7.5 | 14.2 |
| **7** | 14PVAH-40 | 8.4 | 3.08 | 5.7 | 16.1 |
| **8** | 14PVAH-9 | 8.3 | 1.09 | 8.0 | 15.3 |
| | | **Control Hybrids** | | | |
| | DKC80-40BRGEN | 8.2 | 1.64 | 8.0 | 13.7 |
| | 11C1774 | 8.0 | 1.38 | 5.0 | 13.5 |
| | 11C1483 | 6.6 | 1.38 | 8.5 | 14.1 |
| | | **Statistics:** | | | |
| | **Mean** | 7.1 | 1.40 | 6.9 | 14.4 |
| | **Min** | 4.1 | 0.82 | 3.7 | 12.8 |
| | **Max** | 10.1 | 3.08 | 8.7 | 16.2 |
| | **SED** | 0.7 | 0.23 | 0.7 | 0.5 |
| | **CV %** | 14.4 | 23.80 | 13.8 | 5.1 |
| | **LSD (0.05)** | 2.1 | 0.64 | 1.9 | 1.5 |
| | **F (pr.)** | <0.001 | <.001 | <.001 | <.001 |

EPP = ears per plant, EA = ear aspect score (1= very bad; 10 = very good), GMC = grain moisture content.

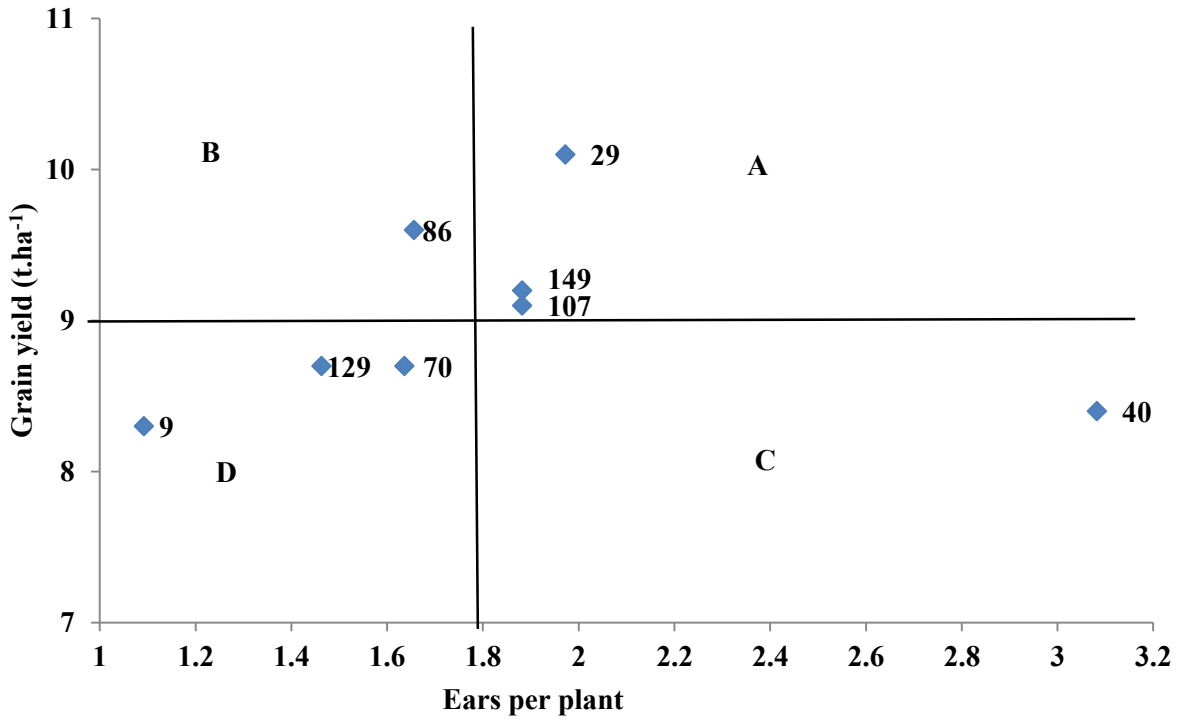


Figure 4.1: Hybrid distribution at Cedara with respect ears per plant and grain yield (Hybrid names are abbreviated by omitting the prefix 14PVAH-, as in Table 4.2)

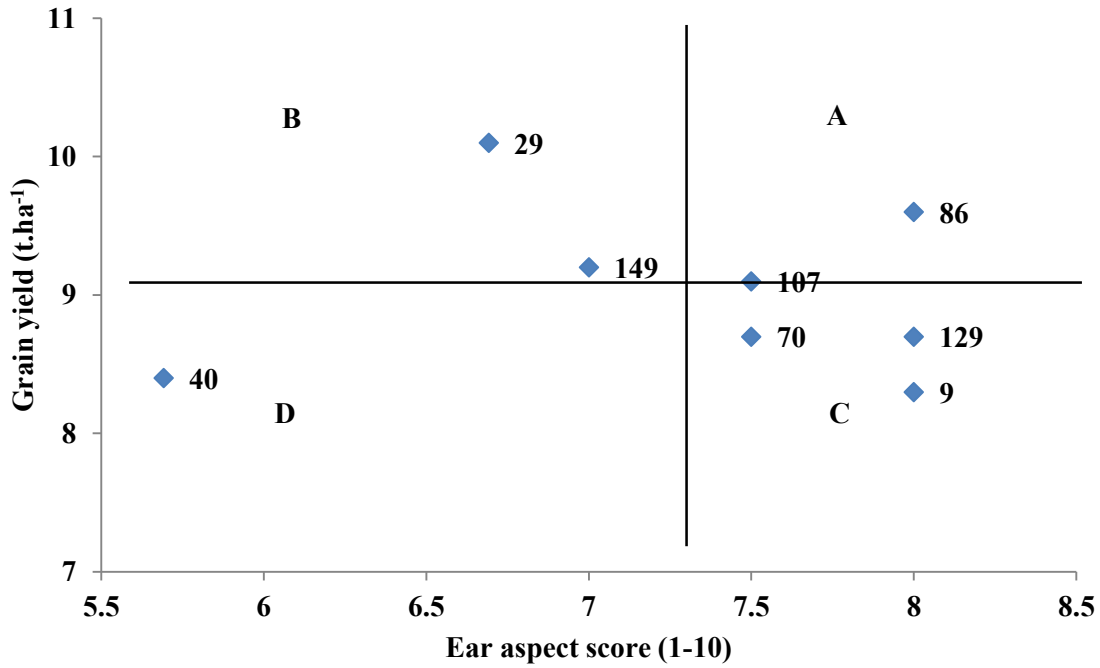


Figure 4.2: Hybrid distribution at Cedara with respect to ear aspect score and grain yield (Hybrid names are abbreviated by omitting the prefix 14PVAH-, as in Table 4.2)

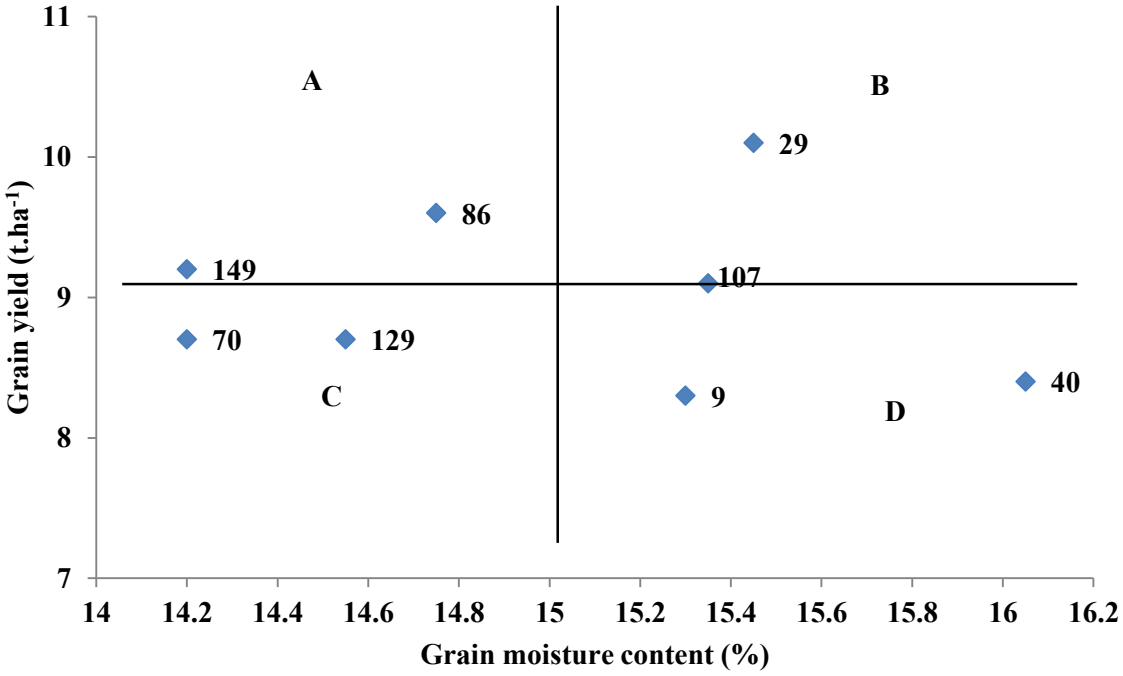


Figure 4.3: Hybrid distribution at Cedara with respect to grain moisture content and grain_yield (Hybrid names are abbreviated by omitting the prefix 14PVAH-, as in Table 4.2)

### 4.3.2 Dundee

Results presented in Table 4.3 shows that all the top 10% hybrids performed the same based on LSD. However, they were significantly higher than the non-selected hybrids. The control hybrid DKC80-40BRGEN had higher yield compared to all top 10% of selected experimental hybrids, and the other two control hybrids were surpassed by all top 10% experimental hybrids. However, the difference was not significantly different based on LSD. The yield and ears per plant were highly significantly different ($p<.001$), and significantly different ($p=0.004$) for grain moisture content. The CVs were less than 16%.

The results presented in Figure 4.4 shows distributions of hybrids, where the hybrids in quadrant labeled A are the most desirable due to both high yield and prolific. Only three hybrids were distributed within quadrant A, and among the three, hybrid 14PVAH-166 and 14PVAH-165

share both male and female parent in a reciprocal mode. However, different situation occurs with hybrid 14PVAH-167 and 14PVAH-168, they share both male and female parent in a reciprocal mode but distributed in two different quadrants mainly due to differences in number of ears per plant. Experimental hybrid 14PVAH-107 can be selected for advancement due to its yield despite its low prolificacy. The results presented on Figure 4.5 shows that only two hybrids were distributed within the desirable quadrant A. Hybrid 14PVAH-28 was the least yielding hybrid and also had the highest grain moisture content.

Table 4.3: Hybrid rank at Dundee with respect to mean grain yield and economic traits

| | Adjusted Yield (t ha$^{-1}$) | | Economic Traits | |
|---|---|---|---|---|
| **Rank** | **Hybrid name** | **Mean yield** | **EPP** | **GMC (%)** |
| | **Top 8 Experimental Hybrids** | | | |
| **1** | 14PVAH-166 | 11.3 | 1.68 | 13.8 |
| **2** | 14PVAH-139 | 10.8 | 1.60 | 14.4 |
| **3** | 14PVAH-165 | 10.8 | 1.59 | 13.5 |
| **4** | 14PVAH-107 | 10.7 | 1.04 | 14.5 |
| **5** | 14PVAH-167 | 10.4 | 1.62 | 13.1 |
| **6** | 14PVAH-168 | 10.1 | 1.09 | 13.6 |
| **7** | 14PVAH-127 | 9.9 | 1.15 | 14.3 |
| **8** | 14PVAH-28 | 9.9 | 1.39 | 15.8 |
| | **Control Hybrids** | | | |
| | DKC80-40BRGEN | 12.0 | 1.83 | 13.0 |
| | 11C1483 | 9.2 | 1.34 | 13.1 |
| | 11C1774 | 9.1 | 1.33 | 13.9 |
| | **Statistics:** | | | |
| | **Mean** | 8.3 | 1.40 | 14.1 |
| | **Min** | 4.6 | 0.87 | 12.8 |
| | **Max** | 11.9 | 2.07 | 16.0 |
| | **SED** | 0.9 | 0.15 | 0.6 |
| | **CV %** | 15.7 | 15.70 | 5.7 |
| | **LSD (0.05)** | 2.6 | 0.43 | 1.6 |
| | **F (pr.)** | <.001 | <.001 | 0.004 |

EPP = ears per plant, GMC = grain moisture content

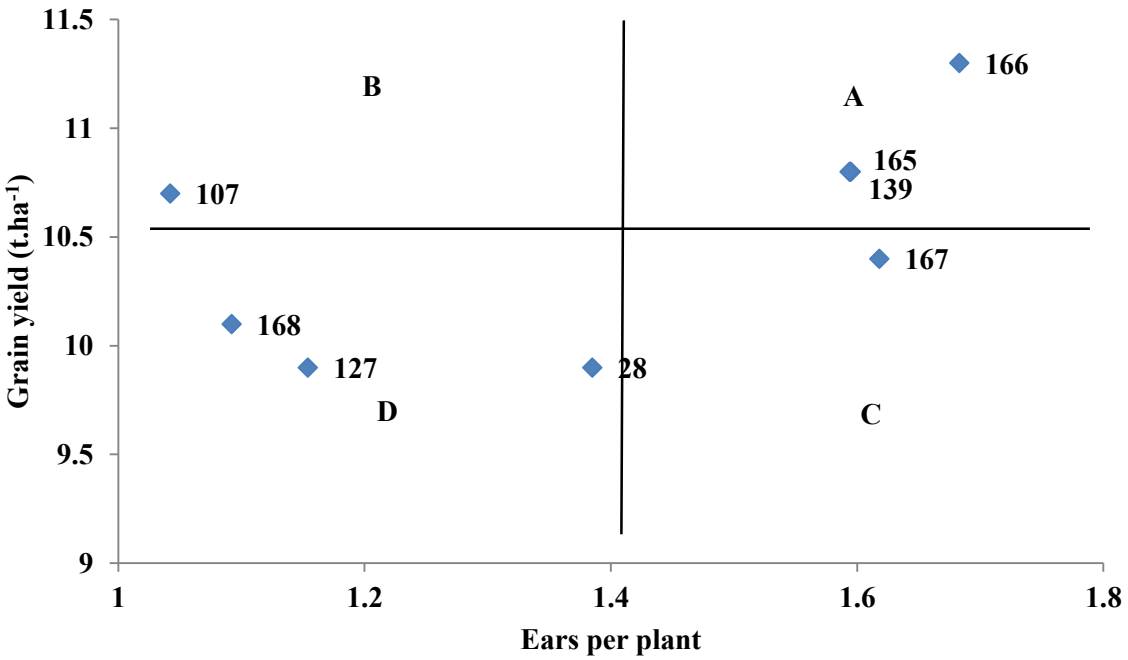


Figure 4.4: Hybrid distribution at Dundee with respect ears per plant and grain yield (Hybrid names are abbreviated by omitting the prefix 14PVAH-, as in Table 4.3)

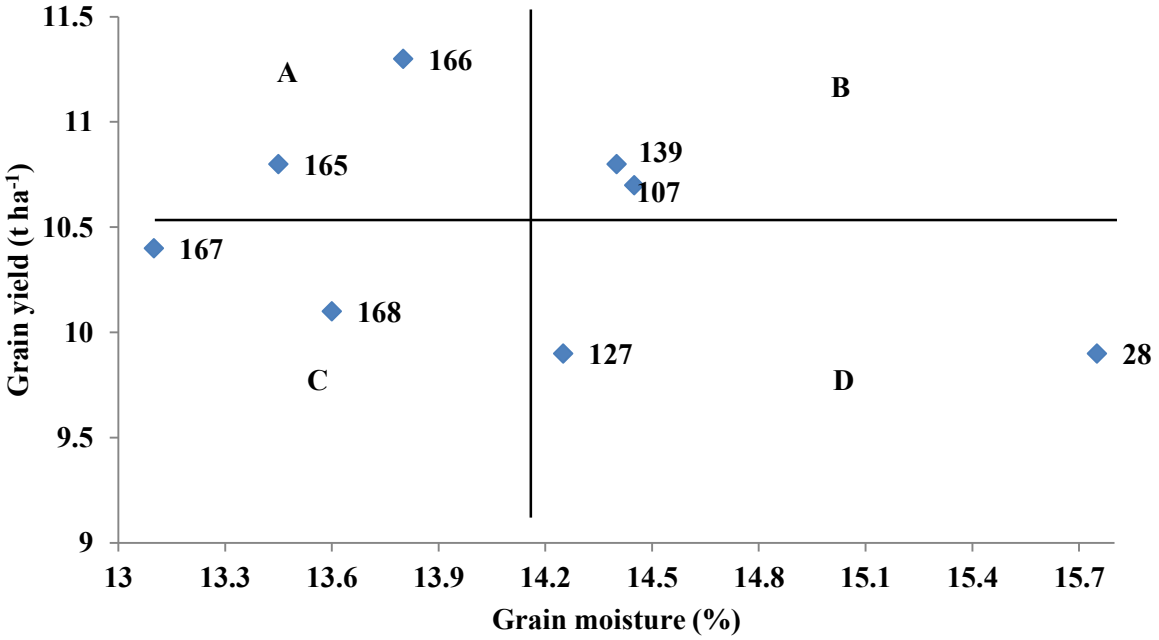


Figure 4.5: Hybrid distribution at Dundee with respect to grain moisture content and grain yield (Hybrid names are abbreviated by omitting the prefix 14PVAH-, as in Table 4.3)

### 4.3.3 Jozini

Table 4.4 shows that all experimental hybrids performed better than all control hybrids in terms of mean grain yields. However, based on LSD only hybrid 14PVAH-8 and 14PVAH-10 performed better than all control hybrids. Both economic traits presented below are significantly different. Figure 4.6 shows hybrid 14PVAH-8 and 14PVAH-10 which are distributed in quadrant A to be high yielding and have high number of ears per plant. Figure 4.7 shows that no hybrid was distributed in the desirable quadrant A, the highest yielding hybrid was among the three hybrids with highest grain moisture content, and the low yielding hybrids had the lowest grain moisture content.

Table 4.4: Hybrid rank at Jozini with respect to mean grain yield and economic traits

| | Adjusted Yield (t ha$^{-1}$) | | Economic Traits | |
|---|---|---|---|---|
| **Rank** | **Hybrid name** | **Mean yield** | **EPP** | **GMC (%)** |
| | **Top 8 Experimental Hybrids** | | | |
| **1** | 14PVAH-8 | 8.2 | 1.21 | 17.5 |
| **2** | 14PVAH-10 | 7.6 | 1.18 | 16.8 |
| **3** | 14PVAH-39 | 6.7 | 0.98 | 17.9 |
| **4** | 14PVAH-59 | 6.7 | 1.03 | 17.8 |
| **5** | 14PVAH-119 | 6.6 | 1.64 | 15.8 |
| **6** | 14PVAH-47 | 6.6 | 1.12 | 16.2 |
| **7** | 14PVAH-67 | 6.4 | 0.97 | 16.2 |
| **8** | 14PVAH-52 | 6.4 | 1.04 | 16.0 |
| | **Control Hybrids** | | | |
| | DKC80-40BRGEN | 4.8 | 1.17 | 14.6 |
| | 11C1483 | 4.7 | 0.98 | 15.5 |
| | 11C1774 | 4.5 | 1.00 | 15.8 |
| | **Statistics:** | | | |
| | **Mean** | 5.3 | 1.10 | 16.6 |
| | **Min** | 3.5 | 1.64 | 19.3 |
| | **Max** | 8.2 | 0.77 | 14.6 |
| | **SED** | 1.1 | 0.18 | 0.9 |
| | **CV %** | 21.4 | 17.3 | 5.4 |
| | **LSD (0.05)** | 2.3 | 0.36 | 1.8 |
| | **F (pr.)** | 0.4 | 0.02 | 0.01 |

EPP = ears per plant, GMC = grain moisture content.

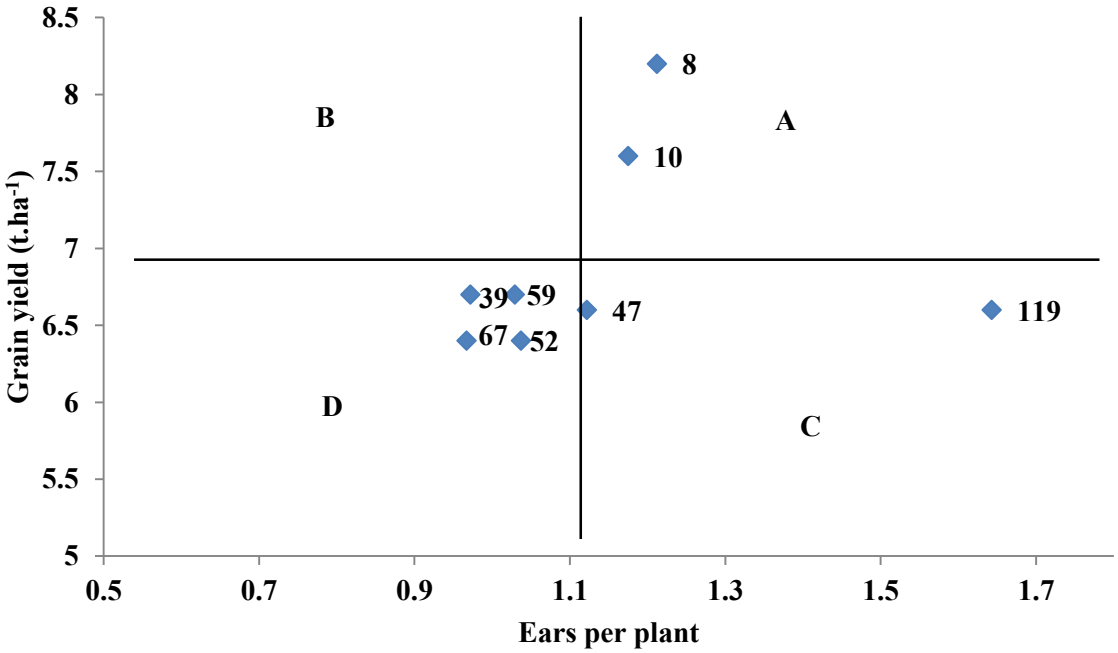


Figure 4.6: Hybrid distribution at Jozini with respect ears per plant and grain yield (Hybrid names are abbreviated by omitting the prefix 14PVAH-, as in Table 4.4)

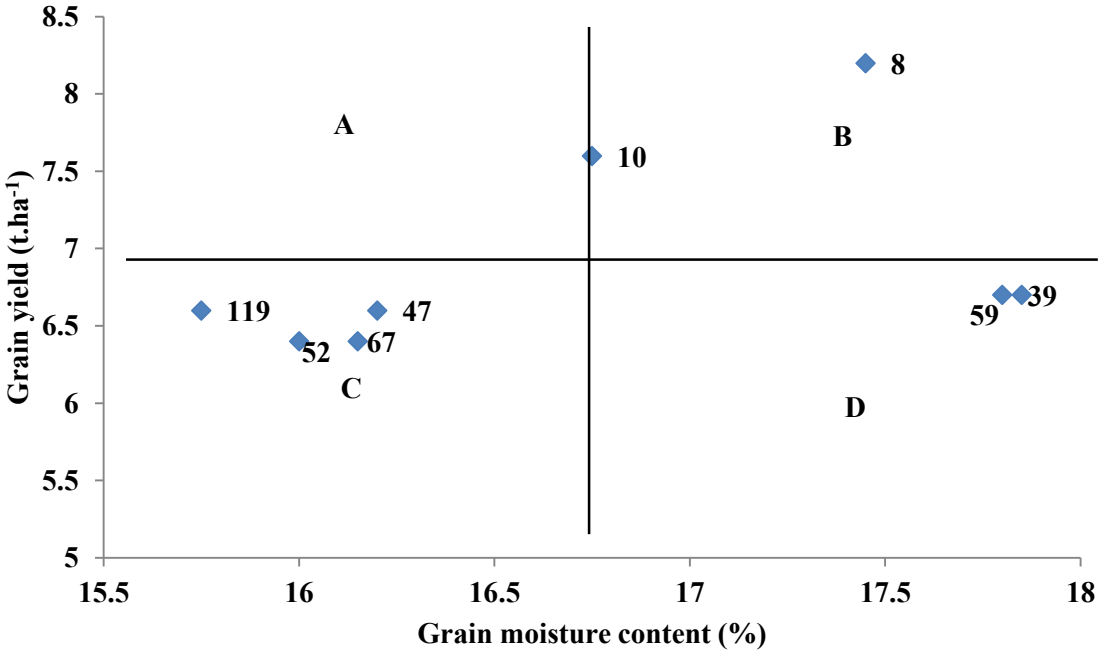


Figure 4.7: Hybrid distribution at Jozini with respect to grain moisture content and grain_yield (Hybrid names are abbreviated by omitting the prefix 14PVAH-, as in Table 4.4)

### 4.3.4 Ukulinga

Table 4.5 shows that the grain yield was not significantly different among the selected experimental hybrids and the checks based on LSD. Economic traits showed significant ($p<0.05$) difference. In Figure 4.8, the association of prolificacy and yield is visible where quadrant A has the most high yielding and higher prolificacy hybrids. Figure 4.10 shows very high grain moisture content compared to other sites, however five of the eight experimental hybrids are lower than the mean, and quadrant A shows the best three high yielding hybrids (14PVAH-50, 14PVAH-10, and 14PVAH-165).

Table 4.5: Hybrid rank at Ukulinga with respect to mean grain yield and economic traits

| Adjusted Yield (t ha$^{-1}$) | | | Economic Traits | | |
|---|---|---|---|---|---|
| **Rank** | **Hybrid Name** | **Mean yield** | **EPP** | **EA (1-10)** | **GMC (%)** |
| | | **Top 8 Experimental Hybrids** | | | |
| **1** | 14PVAH-50 | 10.4 | 1.20 | 5.0 | 17.3 |
| **2** | 14PVAH-10 | 9.8 | 1.25 | 4.5 | 17.6 |
| **3** | 14PVAH-165 | 9.3 | 1.41 | 4.0 | 17.9 |
| **4** | 14PVAH-178 | 9.2 | 1.68 | 5.5 | 17.5 |
| **5** | 14PVAH-194 | 8.9 | 1.03 | 4.0 | 20.6 |
| **6** | 14PVAH-59 | 8.7 | 1.28 | 5.5 | 17.7 |
| **7** | 14PVAH-195 | 8.7 | 1.00 | 4.0 | 18.8 |
| **8** | 14PVAH-109 | 8.7 | 1.15 | 3.0 | 17.7 |
| | | **Control Hybrids** | | | |
| | DKC80-40BRGEN | 10.6 | 1.68 | 7.5 | 16.9 |
| | 11C1774 | 8.2 | 1.66 | 6.0 | 19.3 |
| | 11C1483 | 8.2 | 1.77 | 5.0 | 17.3 |
| | | **Statistics:** | | | |
| | **Mean** | 7 | 1.30 | 5.0 | 17.4 |
| | **Min** | 4.9 | 0.98 | 7.5 | 14.5 |
| | **Max** | 10.6 | 1.88 | 3.0 | 20.7 |
| | **SED** | 1.8 | 0.23 | 1.4 | 1.3 |
| | **CV %** | 25.8 | 17.80 | 27.4 | 7.3 |
| | **LSD (0.05)** | 3.6 | 0.45 | 2.7 | 2.5 |
| | **F (pr.)** | 0.73 | 0.01 | 0.04 | 0.005 |

EPP = ears per plant, EA = ear aspect score (1= very bad; 10 = very good), GMC = grain moisture content.

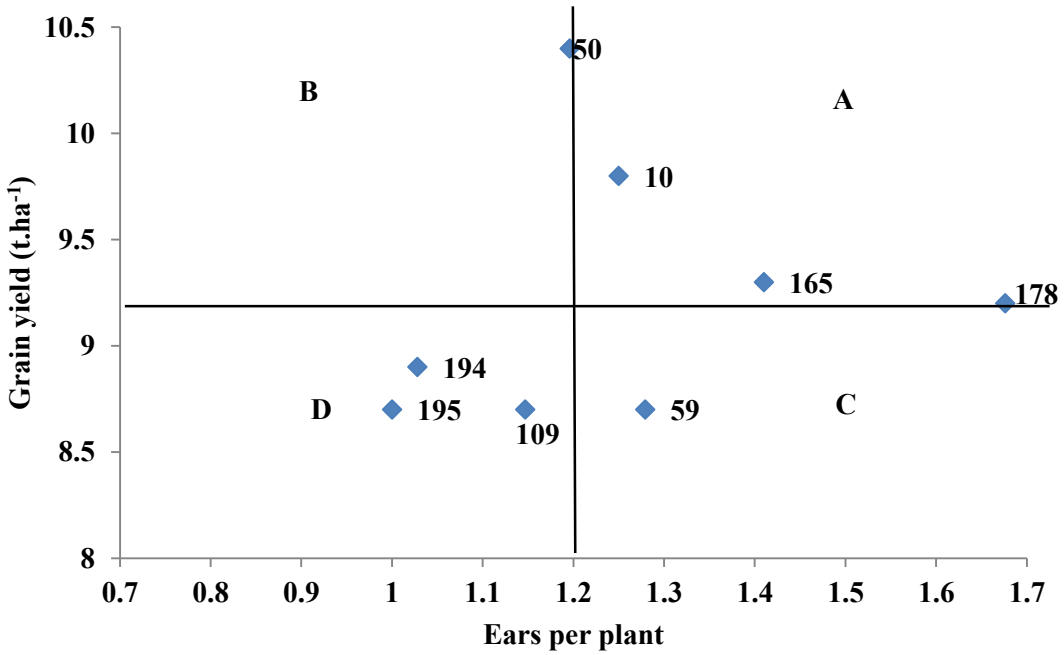


Figure 4.8: Hybrid distribution at Ukulinga with respect ears per plant and grain yield (Hybrid names are abbreviated by omitting the prefix 14PVAH-, as in Table 4.5)

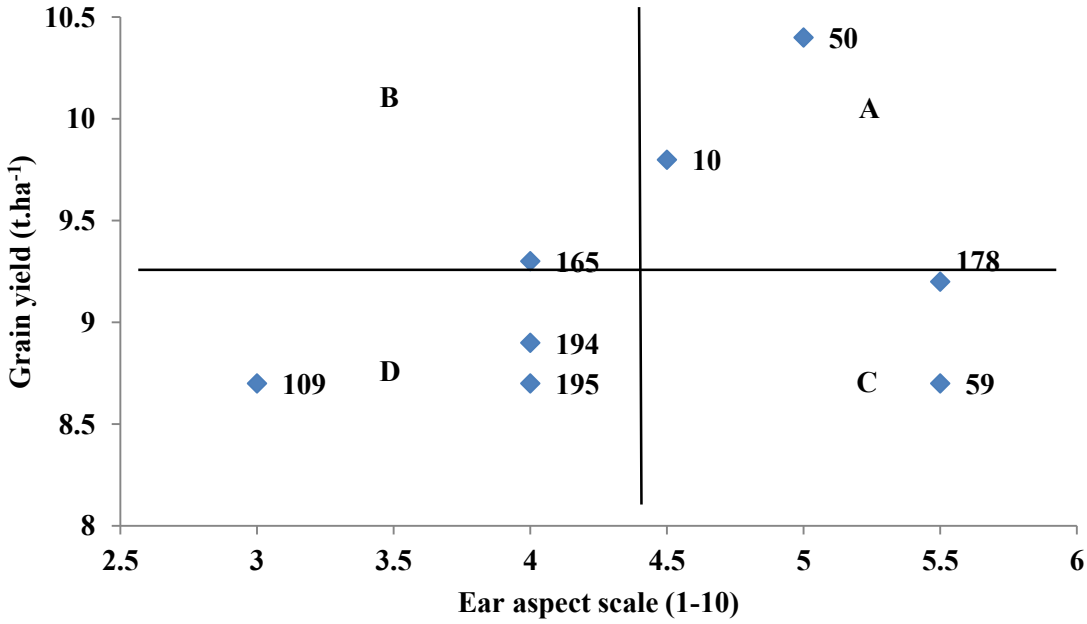


Figure 4.9: Hybrid distribution at Ukulinga with respect to ear aspect score and grain_yield (Hybrid names are abbreviated by omitting the prefix 14PVAH-, as in Table 4.5)

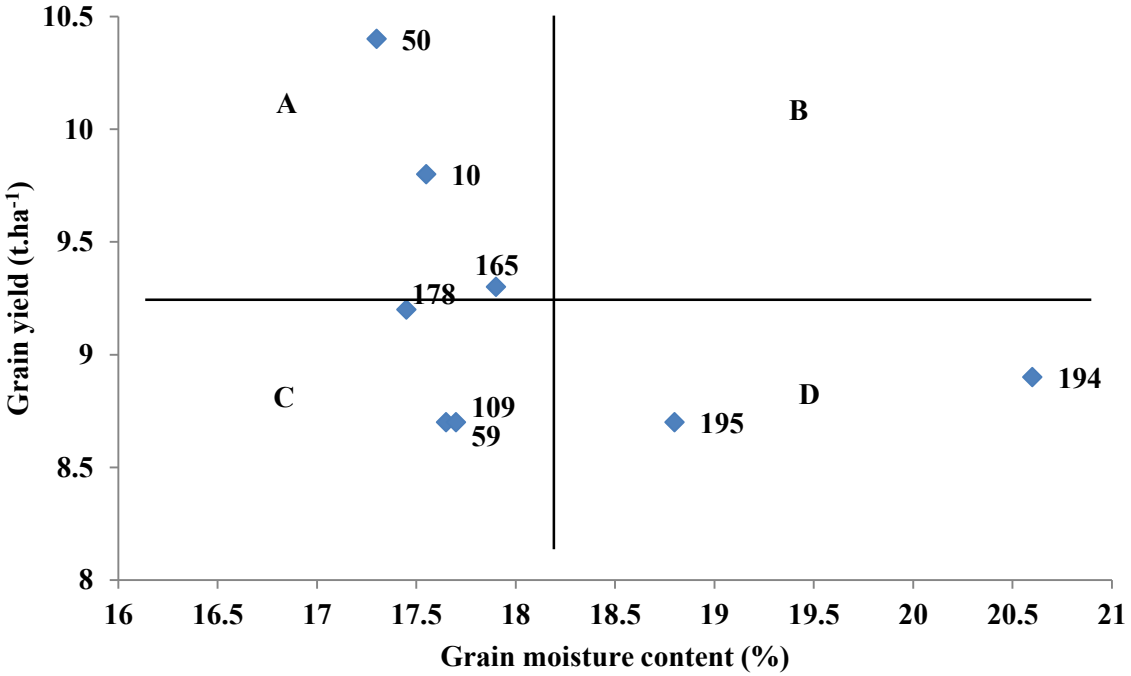


Figure 4.10: Hybrid distribution at Ukulinga with respect to grain moisture content and grain yield (Hybrid names are abbreviated by omitting the prefix 14PVAH-, as in Table 4.5)

The average rank accounts for stability in terms of high yield for hybrids across all for environments. Hybrid 14PVAH-139 was the most stable hybrid while hybrid 14PVAH-188 was the least stable. The experimental hybrids with an average rank of 12, 13, 15, and 17 were more stable and among the check hybrids, DKC80-40BRGEN had an average rank of 18. The other two check hybrids were least stable compared to all eight selected experimental hybrids. The hybrids were highly significantly different ($P < 0.001$) at Cedara and Dundee. However, at Ukulinga and Jozini the hybrids were not significantly different, the CVs were high. Genetic gain calculated through the use of trial means differed throughout the sites to the one calculated through control hybrid means. At Dundee and Ukulinga, control hybrids performed better than experimental hybrids because a negative genetic gain was observed.

Table 4.6: Grain yield means (t $ha^{-1}$) of hybrids evaluated across and within four environments

| Name | Across sites | | Cedara | | Dundee | | Jozini | | Ukulinga | | Average Rank |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rank | Mean | Rank | Mean | Rank | Mean | Rank | Mean | Rank | Mean | |
| **Top 8 Experimental Hybrids** | | | | | | | | | | | |
| **14PVAH-139** | 3 | 8.4 | 14 | 8.1 | 3 | 10.8 | 19 | 5.9 | 13 | 8.1 | **12** |
| **14PVAH-149** | 6 | 8.1 | 3 | 9.2 | 11 | 9.7 | 21 | 5.8 | 18 | 7.8 | **13** |
| **14PVAH-129** | 4 | 8.2 | 5 | 8.7 | 29 | 8.9 | 16 | 6.1 | 10 | 8.6 | **15** |
| **14PVAH-10** | 2 | 8.6 | 36 | 7.3 | 28 | 9.0 | 2 | 7.6 | 3 | 9.8 | **17** |
| **14PVAH-39** | 12 | 7.6 | 11 | 8.2 | 31 | 8.8 | 3 | 6.7 | 38 | 6.9 | **21** |
| **14PVAH-29** | 7 | 8.0 | 1 | 10.1 | 10 | 9.8 | 14 | 6.1 | 64 | 6.0 | **22** |
| **14PVAH-86** | 11 | 7.6 | 2 | 9.6 | 18 | 9.4 | 50 | 5.0 | 22 | 7.6 | **23** |
| **14PVAH-27** | 15 | 7.6 | 12 | 8.1 | 34 | 8.8 | 12 | 6.2 | 35 | 7.1 | **23** |
| **Control Hybrids** | | | | | | | | | | | |
| **DKC80-40BRGEN** | 1 | 8.8 | 10 | 8.2 | 1 | 12.0 | 58 | 4.8 | 1 | 10.6 | **18** |
| **11C1774** | 16 | 7.5 | 15 | 8.0 | 25 | 9.1 | 69 | 4.5 | 11 | 8.2 | **30** |
| **11C1483** | 32 | 7.1 | 57 | 6.6 | 23 | 9.2 | 61 | 4.7 | 12 | 8.2 | **38** |
| **Bottom 5 Experimental Hybrids** | | | | | | | | | | | |
| **14PVAH-11** | 71 | 6.1 | 48 | 6.9 | 58 | 7.4 | 68 | 4.6 | 74 | 5.6 | **62** |
| **14PVAH-194** | 78 | 5.8 | 76 | 5.4 | 80 | 4.7 | 81 | 3.5 | 6 | 8.9 | **61** |
| **14PVAH-186** | 65 | 6.3 | 66 | 6.3 | 52 | 7.9 | 75 | 4.2 | 67 | 5.9 | **65** |
| **14PVAH-151** | 79 | 5.7 | 74 | 5.6 | 71 | 6.8 | 76 | 4.2 | 55 | 6.4 | **69** |
| **14PVAH-188** | 81 | 5.4 | 79 | 5.2 | 79 | 5.1 | 70 | 4.5 | 46 | 6.6 | **69** |
| **Statistics:** | | | | | | | | | | | |
| **Mean** | 6.9 | | 7.1 | | 8.3 | | 5.3 | | 7 | | |
| **Min** | 5.4 | | 4.1 | | 4.6 | | 3.5 | | 4.9 | | |
| **Max** | 8.8 | | 10.1 | | 11.9 | | 8.2 | | 10.6 | | |
| **SED** | 0.94 | | 0.73 | | 0.93 | | 1.14 | | 1.82 | | |
| **LSD (0.05)** | 1.85 | | 2.06 | | 2.61 | | 2.28 | | 3.62 | | |
| **CV %** | 27.1 | | 14.4 | | 15.7 | | 21.4 | | 25.8 | | |
| **F (pr.)** | 0.218 | | <0.001 | | <.001 | | 0.366 | | 0.728 | | |
| **Genetic Gain (%):** | | | | | | | | | | | |
| **$\Delta G_1$** | 31.4 | | 22.0 | | 13.3 | | 16.5 | | 10.5 | | |
| **$\Delta G_2$** | 2.7 | | 14.0 | | -6.9 | | 32.3 | | -14.0 | | |

Average rank = arithmetic mean of rank values across the four environments for the specific hybrid. $\Delta G_1$ is calculated from population mean, and $\Delta G_2$ is calculated from check hybrids mean

The methods used to calculate stability of hybrids in Table 4.7, had different results where hybrid-superiority method had 14PVAH-10 as the top stable hybrid and mean rank method had 14PVAH-139 as the top stable hybrid same as the method used in Table 4.6. In Table 4.7, DKC80-40BRGEN control hybrid is ranked 3rd based on hybrid-superiority method and 5th based on mean rank method, and 14PVAH-10 control hybrid is ranked 1st based on hybrid-superiority method and 4th based on mean rank method. The top 8 hybrids had the lowest superiority value and mean rank, whereas the bottom 5 had the highest superiority value and mean rank values. Hybrids with the lowest superiority value and mean rank also had the highest yield.

Table 4.7: Stability of hybrids measured using cultivar superiority and mean ranks methods

| Name | Cultivar-superiority | Name | Mean rank |
|---|---|---|---|
| **Top 8 Experimental Hybrids** | | | |
| **14PVAH-10** | 1.71 | **14PVAH-139** | 11.25 |
| **14PVAH-139** | 1.90 | **14PVAH-129** | 12.50 |
| **14PVAH-129** | 2.05 | **14PVAH-149** | 14.50 |
| **14PVAH-149** | 2.32 | **14PVAH-10** | 15.75 |
| **14PVAH-165** | 2.73 | **14PVAH-39** | 22.25 |
| **14PVAH-178** | 3.14 | **14PVAH-9** | 23.25 |
| **14PVAH-109** | 3.48 | **14PVAH-29** | 23.25 |
| **14PVAH-9** | 3.53 | **14PVAH-27** | 23.50 |
| **Control Hybrids** | | | |
| **DKC80-40BRGEN** | 2.00 | **DKC80-40BRGEN** | 17.50 |
| **11C1774** | 3.77 | **11C1774** | 29.75 |
| **11C1483** | 4.78 | **11C1483** | 40.25 |
| **Bottom 5 Experimental Hybrids** | | | |
| **14PVAH-31** | 10.31 | **14PVAH-194** | 61.00 |
| **14PVAH-67** | 10.37 | **14PVAH-31** | 61.25 |
| **14PVAH-194** | 11.19 | **14PVAH-11** | 62.25 |
| **14PVAH-188** | 12.07 | **14PVAH-151** | 68.25 |
| **14PVAH-148** | 13.00 | **14PVAH-188** | 68.50 |

The results in Table 4.8 are vital to describe the effectiveness of using the AMMI-2 model to explain the fundamentals of genotype x environment interaction. It can be observed from the table that the genotypes were highly significant different ($p<0.001$), this thereby implies the

possibility to identify best and worst performing genotypes. The residuals were not significant at 5% level of significance therefore it can be concluded that AMMI-2 model was effective to explain genotype by environment interaction.

Table 4.8: The ANOVA table for AMMI model

| Source | df | SS | MS | F | F_prob |
|---|---|---|---|---|---|
| **Total** | **647** | **2338.2** | **3.61** | * | * |
| **Treatments** | 323 | 1664.6 | 5.15 | 2.63 | 0 |
| **Genotypes** | 80 | 321.4 | 4.02 | 2.05 | 0.00001 |
| **Environments** | 3 | 733 | 244.33 | 21.09 | 0 |
| **Block** | 4 | 46.3 | 11.58 | 5.91 | 0.00013 |
| **Interactions** | 240 | 610.2 | 2.54 | 1.3 | 0.01507 |
| **IPCA** | 82 | 280.5 | 3.42 | 1.75 | 0.00036 |
| **IPCA** | 80 | 208.7 | 2.61 | 1.33 | 0.04511 |
| **Residuals** | 78 | 121 | 1.55 | 0.79 | **0.89234** |
| **Error** | 320 | 627.3 | 1.96 | * | * |

Residual is not significant at 5% level of significance, therefore AMMI-2 model is adequate for the data.

The results presented in Table 4.9. The AMMI model revealed Dundee as the highest yielding environment and Jozini was the lowest yielding environment. The hybrid 14PVAH-10 was ranked 1st at Jozini and 3rd at Ukulinga, hybrid 14PVAH-165 was ranked 4th at both Dundee and Ukulinga, and the control hybrid DKC80-40BRGEN was ranked 1st at both Ukulinga and Dundee.

Table 4.9: The AMMI model's first four hybrid selections per environment evaluated

| Sites | Mean (t ha$^{-1}$) | IPCA Score | Hybrid Rank | | | |
|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 |
| **Ukulinga** | 7.002 | 1.974 | DKC80-40BRGEN | 14PVAH-50 | 14PVAH-10 | 14PVAH-165 |
| **Jozini** | 5.321 | 1.073 | 14PVAH-10 | 14PVAH-8 | 14PVAH-129 | 14PVAH-29 |
| **Cedara** | 7.112 | -0.487 | 14PVAH-29 | 14PVAH-107 | 14PVAH-149 | 14PVAH-39 |
| **Dundee** | 8.307 | -2.561 | DKC80-40BRGEN | 14PVAH-166 | 14PVAH-139 | 14PVAH-165 |

Cedara site had more data collected compared to other site, thereby Table 4.10 presents association of yield with other traits where hybrids were evaluated at Cedara and Table 4.11 present association of yield with other traits for hybrids across four sites. Table 4.10 showed that yield reducing traits ear rot, stem lodging, and root lodging has negative correlation ($r^2$) values. Number of ears and ear aspect showed a regression ($R^2$) of 27.3%, 12.9%, respectively, and ($p<0.001$). The results presented in Table 4.11 showed that eight of eleven traits evaluated across environment were significant ($p<0.001$) for both correlation (r) and regression ($R^2$). Number of ears showed a regression ($R^2$) of 37.5%.

Table 4.10: Association of yield with other trait where hybrids were evaluated at Cedara

| | **Correlation** | | **Regression** | | | |
|---|---|---|---|---|---|---|
| **Traits** | **r** | **$P_{value}$** | **$R^2$** | **b** | **s.e.** | **$P_{value}$** |
| **No. of Ears** | 0.458 | <0.001 | 27.3 | 0.1439 | 0.0184 | <.001 |
| **Ear Aspect** | 0.3682 | <0.001 | 12.9 | 0.3664 | 0.0785 | <.001 |
| **Ear Rot %** | -0.308 | <0.001 | 10 | -0.0554 | 0.0127 | <.001 |
| **Standing Plants** | 0.3088 | <0.001 | 9.6 | 0.2848 | 0.0669 | <.001 |
| **Moisture Content** | 0.1875 | 0.026 | 6.2 | 0.359 | 0.105 | <.001 |
| **Ear Per Plant** | 0.1438 | 0.0888 | 5.1 | 0.774 | 0.249 | 0.002 |
| **Stem Lodging** | -0.1866 | 0.0267 | 4.9 | -0.313 | 0.102 | 0.003 |
| **Shelling %** | 0.1365 | 0.1064 | 3 | 0.0646 | 0.0264 | 0.015 |
| **No. of Plants** | 0.2558 | 0.0022 | 2.9 | 0.2281 | 0.095 | 0.018 |
| **Grey Leaf Spot** | 0.1178 | 0.1642 | 2 | 0.1113 | 0.0537 | 0.04 |
| **Plant Height** | 0.1949 | 0.0206 | 1.5 | 0.01159 | 0.00626 | 0.066 |
| **Ear Length** | 0.1238 | 0.1435 | 1.2 | 0.0946 | 0.0546 | 0.085 |
| **Turcicum** | 0.0948 | 0.2635 | 0.9 | 0.1177 | 0.0749 | 0.118 |
| **Phaeosphaeria Leaf Spot** | 0.0588 | 0.4882 | 0.7 | 0.0852 | 0.0586 | 0.148 |
| **Ear Position** | 0.0598 | 0.481 | | | | |
| **Anthesis-Silking-Interval** | 0.0251 | 0.7675 | | | | |
| **Days to Mid-Silking** | 0.0617 | 0.4671 | | | | |
| **Days to Mid-Pollen** | 0.0585 | 0.491 | | | | |
| **Ear Height** | 0.0598 | 0.481 | | | | |
| **Grain texture** | -0.0223 | 0.7934 | | | | |
| **Root Lodging** | -0.0044 | 0.9587 | | | | |

$R^2$ = Regression coefficient of determination, r= Correlation coefficient, b= Regression coefficient, and s.e. = standard error

Table 4.11: Association of yield with other trait for hybrids across four different sites

| | Correlation analysis | | Regression analysis | | |
|---|---|---|---|---|---|
| **Traits** | **r** | **$P_{value}$** | **$R^2$** | **b** | **s.e.** |
| **No. of Ears** | 0.6177 | <0.001 | 37.5 | 0.2368 | 0.012 |
| **Ear Per Plant** | 0.5093 | <0.001 | 27.6 | 2.975 | 0.189 |
| **Plant Height** | 0.4041 | <0.001 | 16.6 | 0.03591 | 0.00364 |
| **Days to Mid-Pollen** | 0.3894 | <0.001 | 9.9 | 0.05396 | 0.00635 |
| **Days to Mid-Silking** | 0.3833 | <0.001 | 9.4 | 0.05661 | 0.00684 |
| **Anthesis-Silking-Interval** | -0.2774 | <0.001 | 6.2 | -0.3231 | 0.0489 |
| **Ear Height** | 0.241 | <0.001 | 5.8 | 0.02558 | 0.00461 |
| **Moisture Content** | -0.3435 | <0.001 | 4.2 | -0.2201 | 0.0407 |
| **Ear Position** | 0.063 | 0.166 | 0.2 | 0.0217 | 0.0152 |
| **Ear Length** | -0.0154 | 0.7358 | | | |
| **No. of Plants** | 0.0354 | 0.4369 | | | |

$R^2$ = coefficient of determination, $r^2$= Correlation, b= Regression coefficient, and s.e.= standard error

Table 4.12 results showed that six out of eleven traits analyzed across the environment were highly significantly different ($p<0.001$). The traits: ear height, ear length, ears per plant, ear position, number of ears, plant height, anthesis-silking-interval, days to mid-pollen, days to mid-silking, grain moisture content, and number of plants.

Table 4.12: Summary statistics of secondary traits for hybrids evaluated across sites

| Statistics: | Mean | Minimum | Maximum | SED | CV% | $LSD_{(0.05)}$ | F (pr.) |
|---|---|---|---|---|---|---|---|
| **Anthesis-Silking-Interval** | 0.107 | -1.875 | 1.125 | 0.7439 | 1395.1 | 1.4612 | 0.818 |
| **Days to Mid-Pollen** | 75.88 | 71.13 | 80.63 | 5.849 | 15.4 | 11.488 | 1 |
| **Days to Mid-Silking** | 75.98 | 71.25 | 81 | 5.415 | 14.3 | 10.636 | 1 |
| **Ear Height** | 118.73 | 94.7 | 142.3 | 8.579 | 14.5 | 16.864 | <.001 |
| **Ear Length** | 20.675 | 18.19 | 23.49 | 0.9696 | 9.4 | 1.9046 | <.001 |
| **Ears Pre Plant** | 1.3 | 0.945 | 1.699 | 0.15 | 23.9 | 0.3 | <.001 |
| **Ear Position** | 46.8 | 38.71 | 54.46 | 2.64 | 11.3 | 5.18 | <.001 |
| **Grain Moisture Content** | 15.64 | 14.2 | 17.32 | 0.9042 | 11.6 | 1.776 | 0.738 |
| **Number of Ears** | 20.45 | 15.62 | 27.25 | 2.138 | 20.9 | 4.2 | <.001 |
| **Number of Plants** | 16.279 | 14.75 | 17.37 | 0.7446 | 9.1 | 1.4625 | 0.595 |
| **Plant Height** | 263.92 | 233.7 | 293.3 | 9.734 | 7.4 | 19.136 | <.001 |

## 4.4. Discussion

### 4.4.1 Selection of the best hybrids within the four different sites

In the current study, grain yield was the primary trait used for selecting the best hybrids. However, other economic traits such as grain moisture content, number of ears per plant, and ear aspect score were put into consideration. This is because the listed economic traits are the most desirable traits by South African commercial farmers. However, the recommendations may differ depending on what the produce will be utilized for in the market.

#### 4.4.1.1 Cedara

The results showed relationship between adjusted grain yield and number of ears per plant. The higher the grain yield the higher was the number of ears per plant, except for hybrid 14PVAH-40. The ear aspect score for hybrid 14PVAH-40 was the lowest because the ear aspect score was determined by number of ears per plant and ear size, the low ear aspect score for hybrid 14PVAH-40 was due to small ears produced. The recommended grain moisture content for maize in South Africa is 12.5%, however for both experimental hybrids and control hybrids it was observed that the grain moisture content was higher than the recommended. Late maturity in maize can be associated with ear diseases such as Fusarium ear rot. Haddadi (2014) conducted a

study on late maturing maize hybrids and lines reactions to Fusarium ear rot and concluded that there was a strong association between yield and this disease.

At Cedara, site all control hybrids were out yielded by the selected experimental hybrids in terms of grain yield, but in terms of grain moisture content the control hybrids had a lower moisture content compared to all selected experimental hybrids.

#### 4.4.1.2 Dundee

The results indicated that check hybrid DKC80-40BRGEN out yielded all best experimental hybrids. However, the other two checks were out yielded by all the selected experimental hybrids. Al-Naggar et al., (2011) indicates that grain yield per unit area can be increased by planting modern hybrids that can tolerate increased planting density by retaining their level of prolificacy. This is inaccordance with the current study, where the results showed a relationship between grain yield and prolificacy and the highest yielding had the highest number of ears per plant. These result illustrates the importance of having many small ears over one big ear. Both experimental and check hybrids had grain moisture content which was above the recommended moisture content by the market of South Africa.

The results showed that experimental hybrid 14PVAH-166 was the best hybrid in terms of adjusted grain yield and number of ears per plant, and ranked 3$^{rd}$ in terms of grain moisture content. However the control hybrid DKC80-40BRGEN showed superiority with all presented traits which were; grain, ear prolificacy, and grain moisture content. Experimental hybrids in quadrant B can be selected for advancement due to high yield despite their higher grain moisture content.

#### 4.4.1.3 Jozini

The results at Jozini showed underperformance by all control hybrids where all best experimental hybrids out yielded all control hybrids in terms of grain yield. The level of prolificacy at Jozini site was very poor where the highest prolific hybrid 14PVAH-119 had 1.64 numbers of ears per plant; this resulted in Jozini being the lowest yielding site in the current study where the highest yield was 8.2 t $ha^{-1}$. The poor prolificacy expressed by hybrids at Jozini is in accordance to the literature. Hamidi et al., (2010) indicates that under poor yield environment the level of prolificacy can be decreased. Experimental hybrids performed better than the MONSANTO control hybrid DKC80-40BRGEN at Jozini mainly because this hybrid is not well adapted to the environmental conditions at Jozini which are very hot and dry.

#### 4.4.1.4 Ukulinga

The control hybrid DKC80-40BRGEN was the best performing hybrid at Ukulinga and it out yielded all experimental hybrids. However, the best experimental hybrids out-yielded the other two control hybrids. Hybrid 14PVAH-50 which ranked 1$^{st}$ according to yield was number 5$^{th}$ according to number of ears per plant, which proves that secondary traits do not guarantee grain yield superiority. The ear aspect score was around 5 mainly because ear aspect score was judged according to prolificacy and size of ears. It can be observed from the results that poor prolificacy was attained from all best experimental hybrids. Grain moisture content for both experimental and control hybrids were higher compared to the South African market demands which is 12.5%, thus this indicates late maturity of these hybrids in this site.

### 4.4.2 Selection of the best stable hybrids across sites

#### 4.4.2.1 Genotype by environment interaction

The experimental hybrids 14PVAH-10, 14PVAH-139, 14PVAH-129, 14PVAH-165, and 14PVAH-149 were the best performing hybrids across sites. The results revealed genotype x environment interaction where different genotypes were adapted to different sites. To take the control hybrid DKC80-40BRGEN as an example, it was ranked 1$^{st}$ at Dundee and Ukulinga, 10$^{th}$ at Cedara, and 58$^{th}$ at Jozini which indicates that this hybrid is well adapted to high yielding

environmental sites (the first two) but lost yield under disease at Cedara and under the tropical lowland at Jozini. The AMMI results showed a significant interaction (GE). Thus, Rahman et al. (2010) reported that significant GE indicated crossover GE where rank changes for the genotypes from location to location within a year and from year to year across locations. This seemed to be the case with the hybrids evaluated at the four sites in this study.

In the current study AMMI-2 model was adequate like in the previous research done by Balestre et al. (2009). The AMMI model's first four hybrid selections per environments evaluated table showed that the environmental conditions for the different sites were not the same. It can be observed that Dundee was the highest yielding environment followed by Cedara, Ukulinga, and Jozini, respectively. The check hybrid DKC80-40BRGEN was ranked 1$^{st}$ at both Dundee and Ukulinga, these were the higher yielding environments which indicates that hybrid DKC80-40BRGEN is well adapted to favorable environment therefore it is economical to commercial farmers who can afford agricultural inputs. Experimental hybrid 14PVAH-10 was ranked 1$^{st}$ at Jozini and 3$^{rd}$ at Ukulinga indicating that this experimental hybrid is well adapted to poor environments therefore it can be selected for subsistence farming purposes. Experimental hybrid 14PVAH-29 is considered adapted to both favorable and poor environmental conditions due good performance at both Cedara and Ukulinga. Experimental hybrid 14PVAH-165 and 14PVAH-166 are reciprocal hybrids and both performed well at Dundee, 14PVAH-165 also performed well at Ukulinga, thus these hybrids may be considered adapted to favorable environmental conditions.

#### 4.4.2.2 Genotype performance

The three methods used to calculate genotype stability yielded different results especially in terms of rankings. However the same hybrids were observed throughout these three methods. These are the most stable hybrids which will be selected for deployment in many environments represented by these sites. Arithmetic average rank method showed that the experimental hybrids 14PVAH-139, 14PVAH-149, 14PVAH-129, 14PVAH-10, and 14PVAH-39 were the best stable experimental hybrids, respectively. Check hybrid DKC80-40BRGEN was ranked 5$^{th}$ among the

top best experimental hybrids and check hybrids 11CI774, and 11CA483 were ranked below the top 10% of best stable experimental hybrids. It can be observed that the major reason for check hybrid DKC80-40BRGEN to be ranked 5$^{th}$ is due to its rank position which was 58$^{th}$ at Jozini, the lowest yielding site in the current study. This is a lowland site where most of the commercial hybrids were not adapted because they were bred for the mid altitude environments of South Africa. The mean rank method generated from Genstat yielded almost the same results as arithmetic average rank method where the check hybrid DKC80-40BRGEN was ranked 5$^{th}$ with both methods where it is comparable with the best stable experimental hybrids. However, there is slight difference among the rankings of 14PVAH-129 and 14PVAH-149 for 2$^{nd}$ and 3$^{rd}$ rank position. Experimental hybrids 14PVAH-139, 14PVAH-129, 14PVAH-149, 14PVAH-10, and 14PVAH-39 were identified as the best stable experimental hybrids.

The cultivar-superiority method generated from Genstat showed that the experimental hybrids: 14PVAH-10, 14PVAH-139, 14PVAH-129, 14PVAH-149, and 14PVAH-165 were the most stable. Check hybrid DKC80-40BRGEN was ranked 3$^{rd}$ among the top best experimental hybrids and check hybrids 11CI774, and 11CA483 were ranked below the top 8$^{th}$ of best stable experimental hybrids. Experimental hybrids 14PVAH-139, 14PVAH-129, 14PVAH-149, 14PVAH-10, and 14PVAH-39 were selected as best hybrids by at least two stability methods. Experimental hybrids in order were generated from inbred lines with the following genetic distancies (0.45, 0.47, 0.5, 0.36, and 0.41). According to the data Appendix 2, it can be observed that among these hybrids; hybrid 14PVAH-139 and 14PVAH-39 shared DPVA20 QPM inbred line as a parent; hybrid 14PVAH-149, 14PVAH-129 and 14PVAH-10 shared DPVA15 QPM inbred line as a parent; and hybrid 14PVAH-139 and 14PVAH-129 shared DPVA7 PVA inbred line as a parent.

### 4.4.3 Association of yield with other traits

Yield is the most important trait that breeders use to select for best performing genotypes; therefore breeding for traits which are associated with yield is vital. Trait may be associated with yield negatively or positively, and directly and/or indirectly. Cedara was the main research

station for the current study thereby many traits were recorded at Cedara while in other three sites few traits were recorded. Table 3.11 results showed that there is a strong positive association between number of ears and yield and the results were highly significant, this explains that many small ears result into a higher yield than one big ear. This means that breeders would rather breed for prolificacy as first priority and then consider breeding for bigger ears as the breeding programme advance. The results showed plant height to have a strong positive association with yield. This can be mainly explained by the fact that the taller the maize plant the more are leaves which will undergo photosynthesis and therefore more energy for processes which are required for yield such as grain filling. Ear aspect was not recorded in all four sites, therefore cannot be evaluated for across sites. However, it can be shown from Cedara site that there was a strong positive association between ear aspect and yield. D'andrea et al. (2014) phenotyped 23 traits related to phenology, light capture, biomass production and partitioning, numerical components of plant grain yield and N metabolism and concluded that the phenotyping was relevant for genetic studies aimed at establishing associations with molecular markers used for assisting crop breeding. Taller maize plants were identified as advantageous for higher grain yield during the phenotyping (D'andrea et al., 2014).

Some traits recorded at Cedara site showed negative association with yield and those traits were ear rot which showed a strong association and stem lodging. Root lodging also showed negative association, however the results were not significant. Rotten ears are very light in weight compared to a normal healthy ear, thus this affects yield negatively. Stem and root lodging are known and proven by the literature to have negative effect on yield (Meister et al., 2014).

### 4.5 Conclusion

One of the objectives in the study was to compare hybrid performance within and across four environments based on yield and economic traits. The results indicated a variation among the four sites in terms of hybrid performance. Variation among the top 10% hybrids in terms of hybrid stability identified using three different methods was observed. Cultivar-Superiority method ranked experimental hybrids as follows, 14PVAH-10, 14PVAH-139, 14PVAH-129,

14PVAH-149, and 14PVAH-165, respectively. Arithmetic average rank method ranked experimental hybrids as follows, 14PVAH-139, 14PVAH-149, 14PVAH-129, 14PVAH-10, and 14PVAH-39, respectively. The mean rank method generated from Genstat ranked experimental hybrids as follows, 14PVAH-139, 14PVAH-129, 14PVAH-149, 14PVAH-10, and 14PVAH-39, respectively. Hybrid 14PVAH-139, 14PVAH-129, 14PVAH-149, and 14PVAH-10 were identified by all three methods in the top 10% stable hybrids, thus they are considered as the most stable hybrids. Furthermore, the genetic distancies were as follows; 0.45, 0.47, 0.5, and 0.36 which means they were above the average. Quality protein maize inbred line DPVA15 was a parent to hybrid 14PVAH-149, 14PVAH-129, and 14PVAH-10, and PVA inbred line DPVA7 was a parent to hybrid 14PVAH-139 and 14PVAH-129. The current study showed that traits such as number of ears per plant, plant height, and ear aspect can be selected in a maize breeding programme because they are strongly and positively associated with yield. The study also indicated that traits such as stem lodging, root lodging, and ear rot must be seriously taken into account because they are negatively associated with yield.

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# CHAPTER FIVE

# GENERAL OVERVIEW OF THE RESEARCH AND THE WAY FOWARD

## 5.1 Introduction

This chapter summarizes the main objectives of the study, highlights the main findings and the implications and recommendations for future research purposes:

The specific objectives of the study were as follows:

- To analyze the genetic diversity present among the Pro-Vitamin A, Quality Protein Maize and Normal Maize lines.
- To identify potential heterotic groups among the Pro-Vitamin A, Quality Protein Maize and Normal Maize lines.
- Identify the top 10% hybrids in terms of hybrid stability.
- Identify traits associated with yield of hybrids among Pro-Vitamin A and QPM lines.

## 5.2 Major findings

The main findings on genetic characterization, cultivar superiority, and accuracy of predicted superior hybrids are presented below.

### 5.2.1 Genetic characterization

- Genetic variability was observed among the twenty inbred lines, the genetic distances ranged from 0.11 to 0.54 between the lines and the majority of the genetic distances were above 0.3.
- Inbred lines were classified into six different clusters, the results showed that inbred lines which appeared in the same cluster were sharing the same previous group named using shared characteristic and origin.
- Each cluster represented a potential heterotic group, therefore it can be concluded that genetic diversity exist and there is a potential of producing superior hybrids.

- It was observed from the study that inbred lines from similar groups as indicated in the study tend to be in similar clusters and those from different groups were observed in different clusters.

### 5.2.2 Best hybrids

The study identified superior hybrids which should be advanced in the breeding programme:

- The AMMI ANOVA showed genotype x environment interaction to be significant at 5% level of significance.
- Experimental hybrids 14PVAH-139, 14PVAH-129, 14PVAH-149, 14PVAH-10, and 14PVAH-39 were identified as the best stable experimental hybrids. Hybrids 14PVAH-139, 14PVAH-129, 14PVAH-149, and 14PVAH-10 performed better than the check hybrid DKC80-40BRGEN, and all the top eight experimental hybrids performed better than 11C1774 and 11C1483 check hybrids.
- The current study showed that traits such as number of ears per plant, plant height, and ear aspect are strongly associated positively with yield, it also showed trait such as stem lodging, root lodging, and ear rot to be associated with yield negatively

### 5.2.3 Accuracy of predicted superior hybrids from genetic characterization

According to the literature, inbred lines that have highest genetic distance are most likely to result into most desirable $F_1$ hybrid. Appendix 2; has the list of expected hybrid superiority in terms of yield.

- According to Appendix 2; hybrids 14PVAH-88, 14PVAH-98 and 14PVAH-187 were expected to be high yielding. However, neither of the hybrids were among the top yielding hybrids, the hybrids may have other desirable traits but yield is the most important trait that is used by plant breeders.
- Hybrid 14PVAH-149 was ranked 4$^{th}$ in terms of potential yield determined by genetic distance, this hybrid was among the top yielding hybrids identified at Cedara and was also ranked 3$^{rd}$ most stable experimental hybrid. Hybrid 14PVAH-129 was ranked 7$^{th}$ in

terms of potential yield determined by genetic distance, this hybrid was among the top yielding hybrids identified at Cedara and also ranked 2nd among the most stable experimental hybrids.

- The results showed that the relationship of genetic distance and heterosis is still not well understood, it also showed that it is not guaranteed that the higher the genetic distance the higher the yielding $F_1$ hybrid. Experimental hybrids that were expected to be high yielding were not among the top yielding hybrids. Therefore, the current study also shows that the relationship between genetic distance and heterosis is not a simple linear one.

**5.3 Implications and recommendations: - the Way forward**

Climate change impose a great threat to food security, it is predicted that at some point maize will surpass wheat and rice to become number one most grown cereal, this is because maize is adapted to wide range of environments and furthermore it is genetically diverse than any other cereal crop. Malnutrition still persists because of food insecurity; Vitamin A deficiency (VAD) is among the problems of malnutrition, therefore increased productivity of maize varieties which are high in pro-Vitamin A and quality protein is required to address the issue of malnutrion and VAD.

Maize breeders are largely utilizing hybrid vigour (heterosis) to produce hybrids which are high yielding and adaptable to harsh environmental changes. However, the breeding processes of utilizing hybrid vigour requires heterotic grouping to minimize the expenses. Marker-assisted-breeding has been identified as a way forward in identifying heterotic group since conventional breeding (phenotyping) has been found to be time consuming and expensive. Nonetheless, the relationship of genetic distance and hybrid vigour is still not well understood. Therefore, such breeding techniques as a combination can be used to improve maize varieties which are high in pro-Vitamin A and quality protein to supplement malnutrition and vitamin A deficiency.

# Appendices

Appendix 1: Major allele frequency, gene diversity, PIC and inbreeding coefficient mean values for the 87 SNP markers used in the study

| Marker | Major Allele Frequency | Gene Diversity | PIC | Inbreeding coefficient |
|---|---|---|---|---|
| **bt2_2** | 0.7500 | 0.3750 | 0.3047 | 1.0000 |
| **csu1171_2** | 0.5000 | 0.5000 | 0.3750 | 1.0000 |
| **Fea2_1** | 0.8000 | 0.3200 | 0.2688 | 1.0000 |
| **PHM4348_16** | 0.8500 | 0.2550 | 0.2225 | 1.0000 |
| **PZA00136_2** | 0.7000 | 0.4200 | 0.3318 | 1.0000 |
| **PZA00223_2** | 0.5500 | 0.4950 | 0.3725 | 1.0000 |
| **PZA00266_7** | 0.5500 | 0.4950 | 0.3725 | 1.0000 |
| **PZA00309_2** | 0.9000 | 0.1800 | 0.1638 | 1.0000 |
| **PZA00343_31** | 0.8500 | 0.2550 | 0.2225 | 1.0000 |
| **PZA00352_23** | 0.6000 | 0.4800 | 0.3648 | 1.0000 |
| **PZA00455_16** | 0.6500 | 0.4550 | 0.3515 | 1.0000 |
| **PZA00543_12** | 0.9500 | 0.0950 | 0.0905 | 1.0000 |
| **PZA00726_8** | 0.8500 | 0.2550 | 0.2225 | 1.0000 |
| **PZA00827_1** | 0.7000 | 0.4200 | 0.3318 | 1.0000 |
| **PZA00878_2** | 0.8500 | 0.2550 | 0.2225 | 1.0000 |
| **PZA00881_1** | 0.7000 | 0.4200 | 0.3318 | 1.0000 |
| **PZA00920_1** | 0.7000 | 0.4200 | 0.3318 | 1.0000 |
| **PZA00947_1** | 0.6000 | 0.4800 | 0.3648 | 1.0000 |
| **PZA00948_1** | 0.5500 | 0.4950 | 0.3725 | 1.0000 |
| **PZA01142_4** | 0.6000 | 0.4800 | 0.3648 | 1.0000 |
| **PZA01292_1** | 0.9000 | 0.1800 | 0.1638 | 1.0000 |
| **PZA01315_1** | 0.9000 | 0.1800 | 0.1638 | 1.0000 |
| **PZA01342_2** | 0.7500 | 0.3750 | 0.3047 | 1.0000 |
| **PZA01396_1** | 0.5000 | 0.5000 | 0.3750 | 1.0000 |
| **PZA01447_1** | 0.7000 | 0.4200 | 0.3318 | 1.0000 |
| **PZA01735_1** | 0.6500 | 0.4550 | 0.3515 | 1.0000 |
| **PZA01755_1** | 0.7000 | 0.4200 | 0.3318 | 0.7725 |
| **PZA01804_1** | 0.7500 | 0.3750 | 0.3047 | 1.0000 |
| **PZA02019_1** | 0.9500 | 0.0950 | 0.0905 | 1.0000 |
| **PZA02027_1** | 0.6000 | 0.4800 | 0.3648 | 1.0000 |
| **PZA02068_1** | 0.7000 | 0.4200 | 0.3318 | 1.0000 |
| **PZA02113_1** | 0.7143 | 0.4082 | 0.3249 | 1.0000 |
| **PZA02148_1** | 0.5000 | 0.5000 | 0.3750 | 1.0000 |
| **PZA02212_1** | 0.8000 | 0.3200 | 0.2688 | 1.0000 |

| | | | | |
|---|---|---|---|---|
| **PZA02367_1** | 0.9000 | 0.1800 | 0.1638 | 1.0000 |
| **PZA02386_2** | 0.8500 | 0.2550 | 0.2225 | 1.0000 |
| **PZA02450_1** | 0.6000 | 0.4800 | 0.3648 | 1.0000 |
| **PZA02564_2** | 0.9000 | 0.1800 | 0.1638 | 1.0000 |
| **PZA02585_2** | 0.6500 | 0.4550 | 0.3515 | 1.0000 |
| **PZA02589_1** | 0.7000 | 0.4200 | 0.3318 | 1.0000 |
| **PZA02606_1** | 0.5000 | 0.5000 | 0.3750 | 1.0000 |
| **PZA02676_2** | 0.8889 | 0.1975 | 0.1780 | 1.0000 |
| **PZA02683_1** | 0.7000 | 0.4200 | 0.3318 | 1.0000 |
| **PZA02763_1** | 0.6000 | 0.4800 | 0.3648 | 1.0000 |
| **PZA02890_4** | 0.7500 | 0.3750 | 0.3047 | 1.0000 |
| **PZA02916_5** | 0.5500 | 0.4950 | 0.3725 | 1.0000 |
| **PZA02957_5** | 0.9500 | 0.0950 | 0.0905 | 1.0000 |
| **PZA03116_2** | 0.9000 | 0.1800 | 0.1638 | 1.0000 |
| **PZA03182_5** | 0.5000 | 0.5000 | 0.3750 | 1.0000 |
| **PZA03231_1** | 0.9000 | 0.1800 | 0.1638 | 1.0000 |
| **PZA03391_2** | 0.5000 | 0.5000 | 0.3750 | 1.0000 |
| **PZA03395_3** | 0.9500 | 0.0950 | 0.0905 | 1.0000 |
| **PZA03404_1** | 0.6000 | 0.4800 | 0.3648 | 1.0000 |
| **PZA03445_1** | 0.7000 | 0.4200 | 0.3318 | 1.0000 |
| **PZA03474_1** | 0.5500 | 0.4950 | 0.3725 | 1.0000 |
| **PZA03507_1** | 0.7750 | 0.3488 | 0.2879 | 0.8633 |
| **PZA03602_1** | 0.5500 | 0.4950 | 0.3725 | 1.0000 |
| **PZA03644_1** | 0.7250 | 0.3988 | 0.3192 | 0.8805 |
| **PZA03661_3** | 0.6000 | 0.4800 | 0.3648 | 1.0000 |
| **PZA03695_1** | 0.7500 | 0.3750 | 0.3047 | 1.0000 |
| **PZA03733_1** | 0.5000 | 0.5000 | 0.3750 | 1.0000 |
| **PZA03743_1** | 0.7500 | 0.3750 | 0.3047 | 1.0000 |
| **PZB00008_1** | 0.6000 | 0.4800 | 0.3648 | 1.0000 |
| **PZB00068_1** | 0.6250 | 0.4688 | 0.3589 | 0.8984 |
| **PZB00085_1** | 0.6250 | 0.4688 | 0.3589 | 0.8984 |
| **PZB00109_2** | 0.6500 | 0.4550 | 0.3515 | 1.0000 |
| **PZB00232_1** | 0.7500 | 0.3750 | 0.3047 | 1.0000 |
| **PZB00772_1** | 0.5500 | 0.4950 | 0.3725 | 1.0000 |
| **PZB00869_4** | 0.9000 | 0.1800 | 0.1638 | 1.0000 |
| **PZB01042_7** | 0.8500 | 0.2550 | 0.2225 | 1.0000 |
| **PZB01156_2** | 0.9500 | 0.0950 | 0.0905 | 1.0000 |
| **PZB01186_1** | 0.7000 | 0.4200 | 0.3318 | 1.0000 |
| **PZB01358_2** | 0.9500 | 0.0950 | 0.0905 | 1.0000 |
| **PZB01400_1** | 0.8500 | 0.2550 | 0.2225 | 1.0000 |
| **PZB01647_1** | 0.9000 | 0.1800 | 0.1638 | 1.0000 |
| **PZB02017_1** | 0.8500 | 0.2550 | 0.2225 | 1.0000 |
| **PZB02033_2** | 0.9500 | 0.0950 | 0.0905 | 1.0000 |

| | | | | |
|---|---|---|---|---|
| **PZB02155_1** | 0.6000 | 0.4800 | 0.3648 | 1.0000 |
| **PZB02283_1** | 0.9000 | 0.1800 | 0.1638 | 1.0000 |
| **PZB02510_5** | 0.5750 | 0.4888 | 0.3693 | 0.9026 |
| **PZD00022_6** | 0.5000 | 0.5000 | 0.3750 | 1.0000 |
| **PZD00027_2** | 0.7368 | 0.3878 | 0.3126 | 1.0000 |
| **PZD00054_1** | 0.6000 | 0.4800 | 0.3648 | 1.0000 |
| **PZD00072_2** | 0.5750 | 0.4888 | 0.3693 | 0.9026 |
| **sh1_2** | 0.7000 | 0.4200 | 0.3318 | 1.0000 |
| **umc128_2** | 0.8750 | 0.2188 | 0.1948 | 0.7816 |
| **ZHD1_1** | 0.9000 | 0.1800 | 0.1638 | 1.0000 |
| **Mean** | 0.7214 | 0.3613 | 0.2870 | 0.9864 |

Appendix 2: Expected hybrid superiority using genetic distance information

| Rank | Hybrid name | Reciprocal | Female | Male | Genetic Distance |
|---|---|---|---|---|---|
| 1 | 14PVAH-88 | 14PVAH-89 | DPVA5 | DPVA15 | 0.53 |
| 2 | 14PVAH-98 | 14PVAH-99 | DPVA5 | DPVA20 | 0.52 |
| 3 | 14PVAH-187 | 14PVAH-188 | DPVA10 | DPVA16 | 0.51 |
| 4 | 14PVAH-148 | 14PVAH-149 | DPVA8 | DPVA15 | 0.50 |
| 5 | 14PVAH-158 | Not crossed | DPVA8 | DPVA20 | 0.49 |
| 6 | 14PVAH-19 | 14PVAH-20 | DPVA1 | DPVA20 | 0.47 |
| 7 | 14PVAH-128 | 14PVAH-129 | DPVA7 | DPVA15 | 0.47 |
| 8 | 14PVAH-177 | 14PVAH-178 | DPVA9 | DPVA20 | 0.47 |
| 9 | 14PVAH-51 | 14PVAH-52 | DPVA3 | DPVA16 | 0.46 |
| 10 | 14PVAH-169 | 14PVAH-170 | DPVA9 | DPVA16 | 0.46 |
| 11 | 14PVAH-86 | 14PVAH-87 | DPVA5 | DPVA14 | 0.45 |
| 12 | 14PVAH-108 | 14PVAH-109 | DPVA6 | DPVA15 | 0.45 |
| 13 | 14PVAH-130 | 14PVAH-131 | DPVA7 | DPVA16 | 0.45 |
| 14 | 14PVAH-138 | 14PVAH-139 | DPVA7 | DPVA20 | 0.45 |
| 15 | 14PVAH-146 | 14PVAH-147 | DPVA8 | DPVA14 | 0.45 |
| 16 | 14PVAH-167 | 14PVAH-168 | DPVA9 | DPVA15 | 0.45 |
| 17 | 14PVAH-49 | 14PVAH-50 | DPVA3 | DPVA15 | 0.44 |
| 18 | 14PVAH-150 | 14PVAH-151 | DPVA8 | DPVA16 | 0.44 |
| 19 | 14PVAH-194 | 14PVAH-195 | DPVA10 | DPVA20 | 0.44 |
| 20 | 14PVAH-11 | 14PVAH-12 | DPVA1 | DPVA16 | 0.43 |
| 21 | 14PVAH-47 | 14PVAH-48 | DPVA3 | DPVA14 | 0.42 |
| 22 | 14PVAH-90 | 14PVAH-91 | DPVA5 | DPVA16 | 0.42 |
| 23 | 14PVAH-39 | 14PVAH-40 | DPVA2 | DPVA20 | 0.41 |
| 24 | 14PVAH-59 | 14PVAH-60 | DPVA3 | DPVA20 | 0.41 |
| 25 | 14PVAH-110 | 14PVAH-111 | DPVA6 | DPVA16 | 0.41 |

| | | | | | |
|---|---|---|---|---|---|
| 26 | 14PVAH-126 | 14PVAH-127 | DPVA7 | DPVA14 | 0.41 |
| 27 | 14PVAH-185 | 14PVAH-186 | DPVA10 | DPVA15 | 0.41 |
| 28 | 14PVAH-118 | 14PVAH-119 | DPVA6 | DPVA20 | 0.39 |
| 29 | 14PVAH-165 | 14PVAH-166 | DPVA9 | DPVA14 | 0.39 |
| 30 | 14PVAH-7 | 14PVAH-8 | DPVA1 | DPVA14 | 0.38 |
| 31 | 14PVAH-29 | 14PVAH-30 | DPVA2 | DPVA15 | 0.38 |
| 32 | 14PVAH-31 | 14PVAH-32 | DPVA2 | DPVA16 | 0.38 |
| 33 | 14PVAH-71 | 14PVAH-72 | DPVA4 | DPVA16 | 0.38 |
| 34 | 14PVAH-106 | 14PVAH-107 | DPVA6 | DPVA14 | 0.38 |
| 35 | 14PVAH-183 | 14PVAH-184 | DPVA10 | DPVA14 | 0.38 |
| 36 | 14PVAH-69 | 14PVAH-70 | DPVA4 | DPVA15 | 0.37 |
| 37 | 14PVAH-9 | 14PVAH-10 | DPVA1 | DPVA15 | 0.36 |
| 38 | 14PVAH-79 | Not crossed | DPVA4 | DPVA20 | 0.36 |
| 39 | 14PVAH-27 | 14PVAH-28 | DPVA2 | DPVA14 | 0.34 |
| 40 | 14PVAH-67 | 14PVAH-68 | DPVA4 | DPVA14 | 0.29 |