Genetic and economic value of a shuttle breeding programme for enhancing adaptability of tropical maize germplasm in South Africa

By

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Thesis Abstract

Maize is the principal crop for food security and livestock feed in South Africa. It is also an industrial crop and the produce is exported to many countries in the world. Therefore there is high seed demand which prompts competition for breeding productive hybrids. However direct introduction of tropical hybrids into the warm temperate South African environments has not been successful. Competitive advantages can be obtained by implementing a "shuttle breeding" programme whereby part of the breeding is done in Zimbabwe and South Africa to minimise research and production costs. Introgression of temperate germplasm in tropical elite inbred lines can also be pursued to obtain adapted hybrids. The aim of this study was therefore to assess the effectiveness of introgression of temperate germplasm into tropical elite maize inbred lines as a strategy to enhance adaptability of new hybrids to South Africa, and also to determine both breeding and economic value of a "shuttle breeding" programme. To this end, the introgressed inbred lines and their hybrid progenies were evaluated in South Africa to determine the effect of the selection environment on their performance and genetic variation. Both genetic and economic gains were evaluated with a view to make recommendations to the small and medium scale enterprises with interests in the market.

Introgression of temperate germplasm into tropical germplasm elite lines did not disrupt the heterotic groupings because most of the introgressed lines (86%) fitted into known existing heterotic groups. Only 14% of the introgressed lines did not show any inclination to towards the known heterotic clusters of their founder tropical parents. These lines were considered to be new recombinant inbred lines that showed little resemblance with their founder parents. Selection environment did not influence heterotic clustering of the introgressed lines, and genetic diversity was identified among introgressed lines developed in the same environment.

Genetic variation was observed for the major economic traits and heritability of 21% to 91%. The introgression was effective for improving grain yield potential and ear prolificacy. Spearman's rank correlation analysis on grain yield and ear prolificacy data showed significant positive correlation between selection environments such as Ukulinga in South Africa and Kadoma Research Centre in Zimbabwe. Therefore Kadoma Research Centre will be recommended for use in breeding new maize

germplasm lines for South Africa. Correlation among traits showed that ear prolificacy and plant height had significant (P<0.05) direct effects on grain yield thus direct selection of these traits will be emphasised in breeding new hybrids.

Introgression of temperate germplasm into tropical elite maize inbred lines was effective for improving their adaptation to warm temperate environments. Positive genetic gains of 5-58% were realised for grain yield potential and 26-46% for ear prolificacy. Whereas 1% to 37% gains were realised for secondary traits such as plant and ear height, anthesis and silking days there was barely any improvement for root and stalk lodging, and grain moisture content at harvest. However, introgressed lines displayed impressive performance *per se* and *inter se* indicating potential for commercial production. The new inbred line 71-DMLF7_88 combined early physiological maturity, high ear prolificacy and grain yield potential qualifying it as a perfect parent for the warm temperate environments. At least six hybrids were stable and adaptable while four were considered to be ideal genotypes relative to standard commercial hybrids such as PAN6Q445B which is a market leader. The exceptional hybrids, 12C20264, 12C22766, 13XH349 and 11C11774 will be advanced in South Africa.

The study also indicated significant economic gains when a shuttle programme is implemented to breed new hybrids following the introgression strategy. The "Shuttle breeding" programme attained a positive net present value (NPV) of $1, 834, 166. 00. This indicated an increase in shareholder value through an opportunity cost of 17% and 3% relative to conventional breeding programmes which are based in South Africa and Zimbabwe, respectively. Positive NPV and genetic gain achieved using the "shuttle breeding" programme makes it a viable option for small and medium scale seed companies with intention to breed and commercialise competitive products in South African.

In general, the study revealed that introgression of temperate germplasm into tropical elite inbred lines using a "shuttle breeding" programme was effective for enhancing adaptability of tropical germplasm to the South African warm temperate environments.

Declaration

I, Lennin Musundire, declare that:

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

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

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

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Signed……………………………………………………………...

Date…………………………………………………………….

As research supervisors we agree to the submission of this thesis

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Prof John Derera (Supervisor)

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Dedication

This thesis is dedicated to people that have always believed and supported me; wife (Mabel), kids (Tanaya and Tanisha), parents (Nicholas, Leadmore, and Margret), siblings (Florence, Annah, Caroline, and Tonderai) and my late sister (Rhodah).

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List of abbreviations

AD	Anthesis days
AMMI	Additive main effects and multiplicative interaction
ANOVA	Analysis of variance
Av	Average
CBA	Cost benefit analysis
Cedara	Cedara Research Station
CGIAR	Consultative Group on International Agricultural Research
CGV	Coefficient of genotypic variation
CIMMYT	International Centre for Maize and Wheat Improvement
CV	Coefficient of variation
DNA	Deoxyribonucleic acid
EH	Ear height
EPP	Ear prolificacy
Ex-PVP	Ex-plant variety protected
FAOSTAT	Food and Agriculture Organisation Statistics
G	Genotype
GE	Genotype-by-environment interaction
GG	Genetic gain
GGE	Genotype, and genotype-by-environment
GLM	General linear model
GLS	Gray leaf spot
ISSR	Inter simple sequence repeats
K	Potassium

KRC	Kadoma Research Centre
LSD	Least significant difference
M.A.S.L	Meters above sea level
Max	Maximum
MBC	Mean of better check
MC	Moisture content
MCS	Mean of checks
Min	Minimum
MP	Mean of population
MS	Mean of selected
MSV	Maize streak virus
NPV	Net present value
N	Nitrogen
N_3	Salisbury white
NCLB	Northern leaf corn blight
OPV	Open pollinated variety
P	Phosphorous
P	Natal Potchefstroom Pearl Elite Selection
PH	Plant height
PLS	Phaeosphaeria leaf spot
PIC	Polymorphic information content
Potchefstroom	Potchefstroom Research Station

RAPD	Random amplified polymorphic DNA
RARS	Rattray Anorld Research Station
RFLP	Restriction fragment length polymorphism
RL	Root lodging
SADC	Southern African Development Community
SC	Southern Cross
SD	Silking Days
SL	Stalk lodging
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeats
Temp	Temperature
Ukulinga	Ukulinga Research Station
UPGMA	Unweighted Pair Group Method with Arithmetic
°	Degrees
°C	Degree Celsius

Introduction to Thesis

Significance of maize in South Africa

Maize (*Zea mays*, L) is a major staple cereal crop that also has high economic value as a livestock feed in South Africa. Its wide adaptability and usage has resulted in it being the largest locally-produced field crop and an important source of carbohydrate in the South African Customs Union (National Agriculture Marketing Council, 2009). According to the Food and Agriculture Organization of the United Nations Statistics (FAOSTAT, 2012), maize ranks as one the most strategic economic crops in relation to net production in metric tonnes and net production value in Southern Africa, in particular South Africa as depicted in Table 1. It is predicted that the growing South African population will become increasingly dependent on maize for food, feed and industrial usage, with an expected increase in demand for animal feed close to 6.4 million tonnes by 2020 (Syngenta, 2013).

Table 1: Rank, Country, Production (Int $1000) and Production (MT) of Maize in Southern Africa

Rank	Country	Production (Int $1000)	Production (MT)
1	South Africa	846296	10360000
2	Malawi	452601	3699150
3	Zambia	341462	2496430
4	Mozambique	263276	2090790
5	Zimbabwe	175024	1327510
6	Angola	168476	1262220
7	Madagascar	60720	458587
8	DRC	154907	263185
9	Lesotho	9267	73390
10	Swaziland	3072	54857
11	Botswana	1699	16644

Source: (Adjusted after) Food and Agriculture Organization of the United Nations Statistics 2012

Economic importance of South African maize production industry is also extended to regional and international exports. Grain South Africa (2013) reports that South Africa exports maize grain and its by-products, which make at least 24% of its total production, to countries like Botswana, Lesotho, Mauritius, Mozambique, Namibia, Swaziland, Zambia and Zimbabwe in the Southern African Development Community (SADC) region. International exports to countries like Mexico, Italy, Korea, Taiwan

and Japan reflects the importance of the maize industry as a significant earner of foreign currency to the South African economy. Thus any production constraint of maize has potential negative consequences on the economy, health and political stability of South Africa and the SADC region. Breeding programmes operating both inside and outside the South African environment appreciate the potential and lucrativeness of this market. Hence efforts have been made to directly and indirectly breed maize hybrid varieties for this market. However breeding progress has been slow because of poor adaptation of introduced maize germplasm due to effects of climatic changes in the region. Advances in technologies have allowed breeding programmes to reduce some of the effects. This ensures delivery of maize hybrid varieties that have wide adaptability and increased productivity. Therefore the current study focus will be on improving current tropical elite maize inbred lines through incorporation of genes from temperate germplasm to enhance adaptability to South African warm temperate environments. Introgressed lines developed and resultant hybrid varieties will contribute towards increased adaptability of tropical germplasm for increased productivity to the South African environments.

Incorporation of exotic germplasm in tropical maize

The reported slow progress in developing tropical germplasm that is adaptable to the South African environment can be partly attributed to a limited breeding germplasm lines that can fit in its "unique" environmental conditions as shown in Figure 1. South African Government Information (2013) describes the South African environment as unique in the sense that it lies between subtropical locations, on either side of 30° S, accounting for the predominant warm temperate climate. It can be regarded as a transitional environment hence appropriate germplasm should be developed. Xu (2008) classified maize according to latitude and climatic regions grown in namely: tropical maize grown between the tropic of Cancer at about 23° 26' 22" N and tropic of Capricorn approximately 23° 26' 22" S; and temperate maize grown extending north from the tropic of Cancer (at about 23° 26' 22" N latitude) to the Arctic circle at approximately 66° 33' 39" N latitude, and extending south from tropic of Capricorn (at

approximately 23° 26' 22" S latitude) to the Antarctic (at approximately 66° 33' 39" S latitude) as shown in Figure 2.
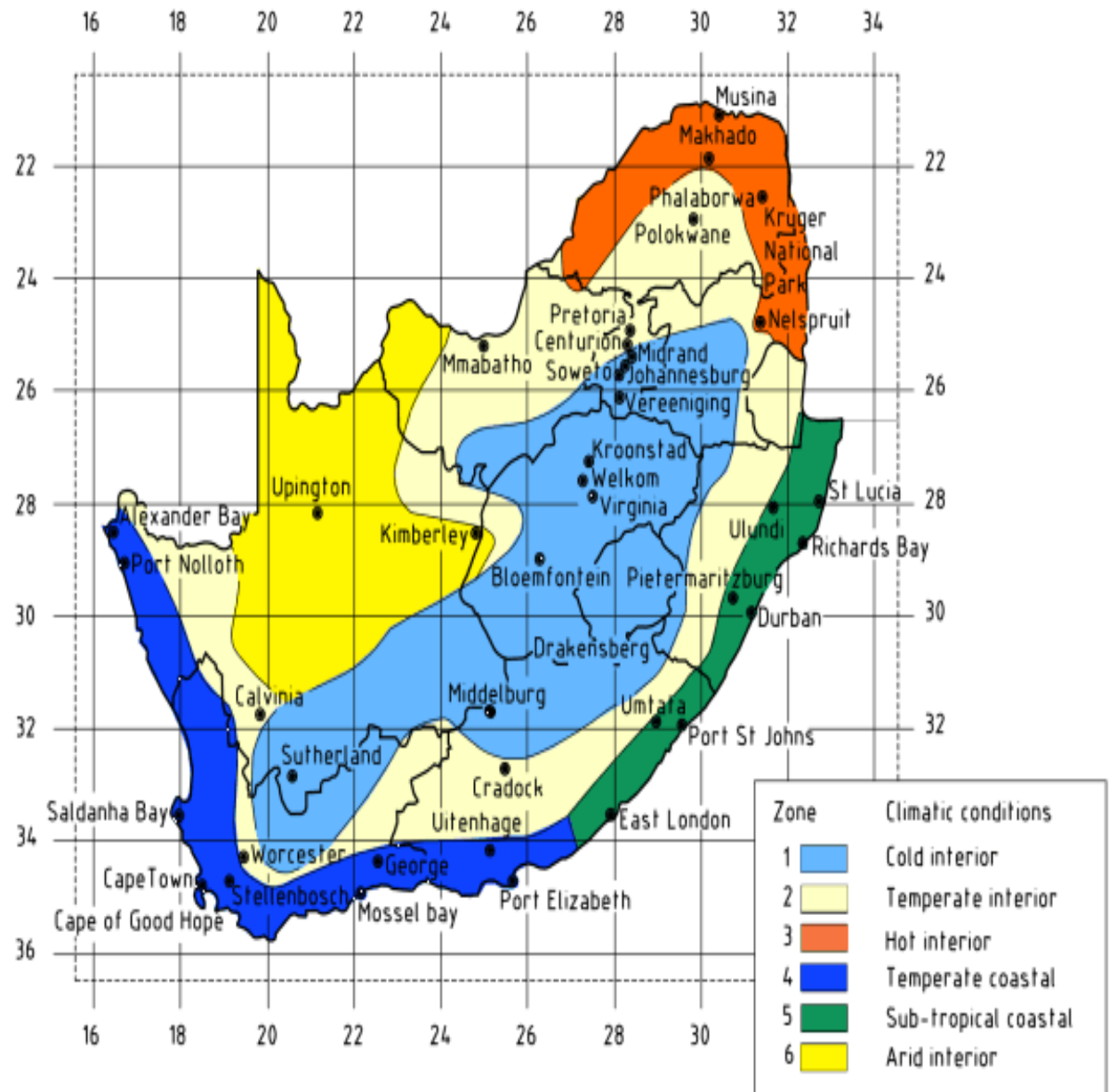
Figure 1: South Africa climate zones (adapted from http://www.insulpro.co.za)
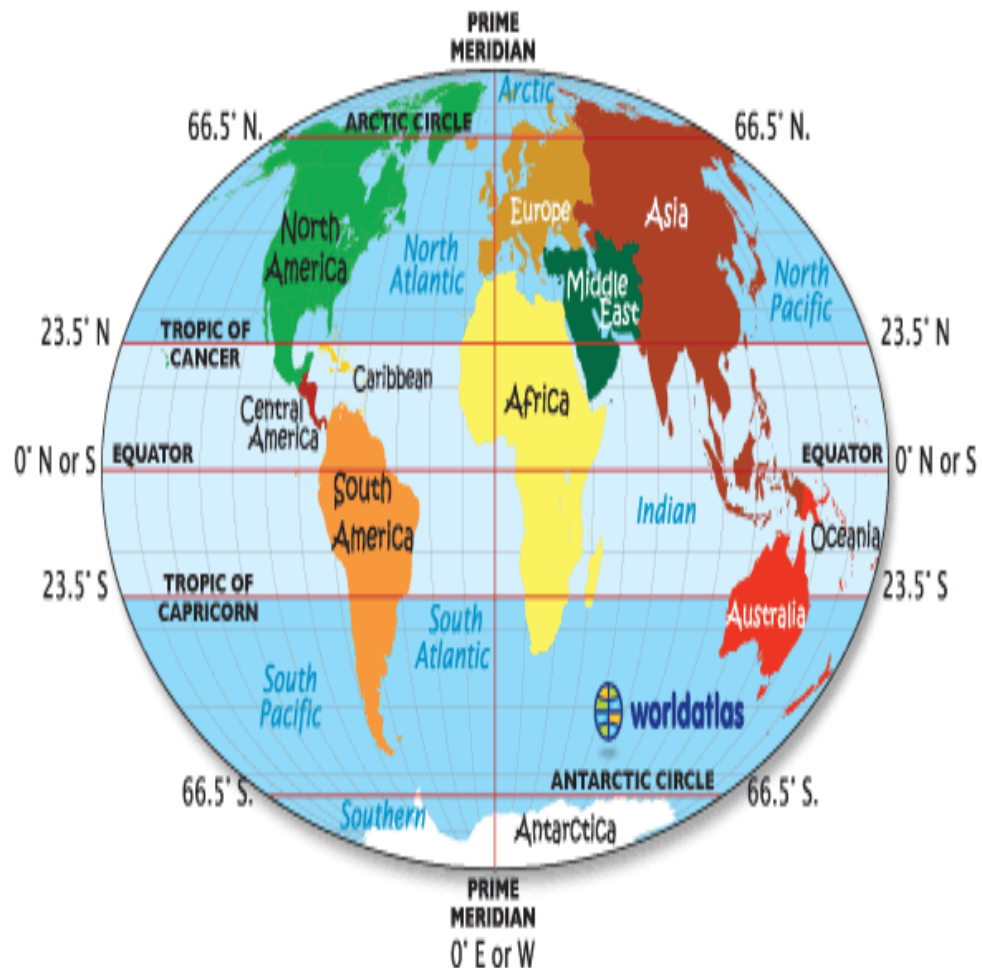
Figure 2: World map showing tropical environments between 23.5°S and 23.5°N of the equator and the temperate zones beyond 23.5°S and 23.5°N (adapted from http://www.worldatlas.com).

Thus, the greater part of South African maize production environments falls under the temperate category, indicating that temperate germplasm should be incorporated in tropical germplasm intended for production in this environment. Further classification has also been made on tropical maize based on altitude of environment grown in: lowland tropics (considered here to be < 1200 m.a.s.l), mid-elevation tropics and subtropics (1200–1800 m.a.s.l), and the highlands (1800–2800 m.a.s.l) (Xu et al., 2009). This classification indicates that tropical maize germplasm represents a diverse source of germplasm and is a valuable natural resource that plays a key role in future maize breeding programmes (Reif et al., 2004).

Exploitation of this genetic diversity of tropical maize germplasm will enhance maize production across diverse environmental conditions of the Southern African region in particular South Africa. However, the South African environment is predominantly warm temperate therefore direct utilization of tropical germplasm has been characterized by a high failure rate of various tropical breeding programmes. Tropical germplasm that is otherwise high yielding and well adapted to tropical environments as depicted in Figure 3, exhibits poor adaptability to the South African temperate environments characterized by: lack of frost tolerance; photoperiod sensitivity; stem and root lodging; poor tassel-silk synchronization; high grain moisture content at harvest; excessive rank growth; poor husk cover; late maturity; low grain yield potential; low harvest index and susceptibility to disease and aphids (Lewis and Goodman, 2003; Tarter et al., 2004; Darsana et al., 2004).

Breeding programmes have also explored the option of direct utilization of previously propriety lines and hybrids (ex-plant variety protected germplasm) from the United States of America that has moved into the public domain when their limited-duration plant variety protection expired (Ex-PVP) (Bretting, 2006). Both private and public breeding programmes have introduced this germplasm into the South African environments. According to Nelson et al. (2008), Ex-PVP lines are temperate germplasm which is a valuable source for breeding of traits of agronomic importance such as good plant standing ability, frost tolerance, early maturity and high yield potential.

However, Nelson et al. (2008) and Andrew (2012) reports that this material has its major weakness in breeding programmes namely: it offers very little in terms of genetic diversity which reduces potential for long term gains in productivity; it is 3 to 6 cycles behind most current elite germplasm and when directly introduced into tropical environments it is highly susceptible to new pathotypes of diseases and pests (Tarter et al., 2004) such as turcicum leaf blight disease and common rust. Farmers who utilize such maize hybrids have to incur an additional cost of fungicide sprays to minimize the yield penalty. This might not be sustainable especially for the smallholder farmers hence the need for a more sustainable option. Incorporation of

genes from exotic germplasm responsible for adaptation to temperate environments into tropical germplasm will be expected to enhance adaptability and productivity in South Africa. However, success of the introgression strategy will be more pronounced with the aid of a suitable and cost effective breeding approach.

		Day length	Mean Temp.	Rainfall
	Dry			≤ 600 mm
(red)	Tropical Lowlands	11≤ d <12.5 hrs	≥ 24 °C	> 600 mm
(teal)	Tropical Midaltitudes			
	Moist	11≤ d <12.5 hrs	18°C ≤ T < 24°C	> 600 mm
	Dry			≤ 600 mm
(yellow)	Subtropical Lowlands	12.5≤ d <13.4 hrs	≥ 24°C	> 600 mm
(green)	Subtropical Midaltitudes			
	Moist	12.5≤ d <13.4 hrs	18°C ≤ T < 24°C	> 600 mm
	Dry			≤ 600 mm
(blue)	Highlands/Temperate	d ≥13.4 hrs **or**	T < 18°C	> 600 mm
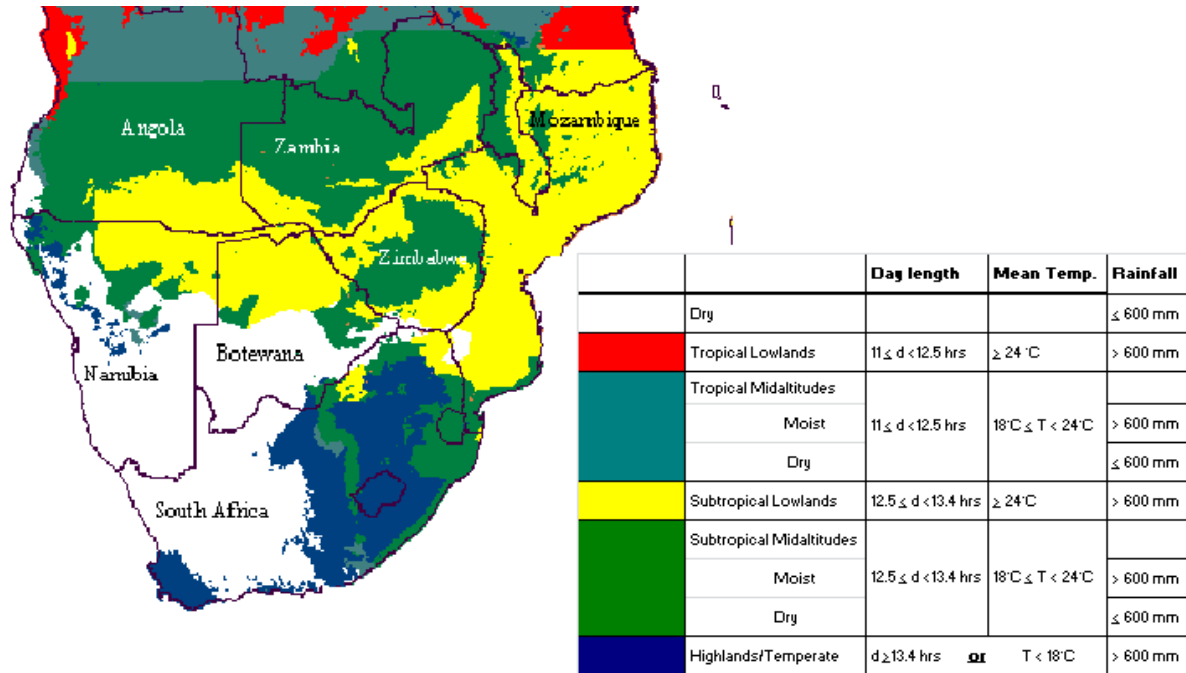
Figure 3: Climatic zones for tropical germplasm programmes (adapted http://www.worldclim.org/)

Breeding strategies

Introgression of tropical germplasm with genes from temperate germplasm for desired economic traits namely: good plant standing ability, low grain moisture content at harvest, ear prolificacy and high grain yield is likely to enhance adaptation and productivity to the South African warm temperate environment. Ear prolificacy which is defined as the ability to produce more than one ear per plant has shown high positive correlations with grain yield potential in maize (du Plessis, 2003). Previous research has reported that prolific maize hybrids have greater efficiency in using environmental resources under stress and greater grain yield stability across environments than non-prolific hybrids (Varga et al., 2004). Hence, the current study

will focus on introgressing ear prolificacy trait in tropical germplasm. However, stalk and root lodging has often been cited in prolific hybrids (Thomison and Jordan 1995) indicating the need for improvement of good standing ability in this germplasm. In addition, high grain moisture content at harvest is characteristic of tropical maize germplasm under temperate growing seasons (Tarter et al., 2004). Therefore the current study will focus on selecting germplasm with low grain mositure content at harvest, an economically important trait for the South African environment. Finally the primary objective in all maize breeding programmes is to develop hybrid varieties that have high yield potential in target environments. Hence, selection for high grain yield potential will also be carried out in this study.

Breeding progress

An efficient and effective breeding programme ensures that genetic improvement in economic traits of interest is accompanied by maintainance of genetic variation. In this study, introgression of temperate germplasm might alter the genetic variation of the introgressed lines hence there will be the need to establish genetic variation for the desired economic traits. According to Bello et al. (2012), the success of any maize breeding programme not only depends on the amount of genetic variability present or created in a population but also on the extent to which it is heritable. Therefore knowledge of the genetic variation and heritability of grain yield and its components will assist in developing breeding strategies and will help to ascertain the real potential of the introgressed lines in hybrid combinations. Smalley et al. (2004) reported that estimation of heritability would also assist in determining resource allocation necessary to effectively select for the trait of interest and to achieve maximum genetic gain with minimum time and resources. Thus it will therefore be important to determine genetic variation and heritability of grain yield and its components in maize introgressed lines to facilitate and sustain an effective breeding programme.

Establishment of breeding progress achieved in improving economic traits of interest is important in ensuring effective progress during selection. According to Al-Tabbal

(2012), estimates of genetic variation and heritability alone do not provide an idea about genetic gain in the next generation but have to be considered in conjunction with estimates of genetic advance achieved. Singh and Chaudhary (2004) reported that genetic advance attained under selection is a measure of genetic gain obtained between the mean of selected progenies and the base population under a specified selection pressure. Genetic gain can either be measured as realised genetic gain, actual achievable gain (Weng et al., 2008) or predicted genetic gain, expected gain in breeding programmes (Callister et al., 2013). Breeding programmes prefer high genetic gain that is associated with high heritability estimates to ensure effective progress during selection (Bello et al., 2012). Therefore, in this study estimate of genetic gain of introgressed lines relative to parental lines will be carried out. Adapted check varieties will be included as controls.

The ultimate objective of breeding programmes is to develop maize germplasm that has wide adaptability and productivity for the target environments. Singh (2006) reported that selection during inbred line development should be effective in improving the performance of inbred lines for their target environments, which is an important factor in hybrid seed production. Therefore, it will be prudent to establish effectiveness of the introgression programme in enhancing tropical inbred lines *per se* performance in the South African warm temperate environments. However, it should be noted that general inbred line performance *per se* is not as important as identification of inbred lines that produce outstanding hybrid performance in hybrid combination, *inter se* performance. Thus, an evaluation of the introgressed lines *inter se* performance will be carried out to establish adaptability and productivity of the tropical germplasm relative to the commercial check hybrids.

Development of hybrid cultivars that are widely adaptable and productive should be complemented with a cost effective and productive implementation of the programme. There is need to have a full consideration of long term sustainability and profitability. Therefore, there will be need to evaluate breeding strategies that will be used in enhancing adaptability of tropical germplasm to the South African environments. In this study, a "shuttle breeding" programme will be utilized to facilitate identification of

introgressed lines that are adaptable to the South African warm temperate environments without compromise to genetic gains. The "shuttle breeding" programme is defined as a breeding methodology involving selecting and advancing of segregating populations in two or more distinct environments to enhance adaptability across varied environments (Oritz et al. 2007). The utility of a "shuttle breeding" programme will be assessed against current approaches for breeding hybrid varieties for South Africa. Current approaches are designated as "conventional breeding programmes".

Conventional breeding

Under conventional breeding there are two possible options namely: setting up a full breeding programme based in South Africa; and the second option involving breeding outside South Africa using tropical maize germplasm followed by introduction and evaluation for adaptability of advanced germplasm in the South Africa warm temperate environments. Under the first option all the three main processes involved in breeding are carried out within South Africa. According to Cleverland and Soleri (2002) the three main process are: ensuring adequate genetic variation and high selection pressure of important traits at all stages of breeding; utilization of experimental procedure to achieve high level of heritability in the breeding trials and yield evaluation trials that achieve a high genetic correlation between performance of germplasm in the breeding trials and under on-farm conditions. This is the ideal approach as both the breeding and yield evaluation trials are carried out in the same environmental conditions enhancing adaptability and productivity. However setting up a breeding programme in South Africa has resulted in high research operational costs which threaten their economic sustainability. The second option has been used by a number of public and private breeding programmes. This has resulted in products which apparently lack desired economic traits for this market. As a result there has been a high failure rate of various breeding programmes operating from tropical environments outside South Africa that have opted to directly introduce their tropical germplasm into the South African temperate environments. Therefore, this implies that a new breeding strategy such as the "shuttle breeding" programme is required to

facilitate identification of adaptable maize hybrids for the South African temperate environments.

"Shuttle breeding"

The "shuttle breeding" approach is not new but its application to developing maize hybrid varieties for South Africa has not been reported in the literature. Ortiz et al. (2007) and Khush (2001) define "shuttle breeding" as a breeding approach in which segregating populations are evaluated in two or more contrasting environments and then tested under near optimum conditions for enhanced productivity across diverse environments. In this study, temperate germplasm will be introgressed into tropical elite inbred lines and the segregating populations will undergo major selection for desired economic traits up to F_2 generation in the South African temperate environments. Resultant F_3 progenies will then be advanced in parallel programmes from F_3-F_5 generation at three distinct environments namely: Kadoma Research Centre and Rattray Anorld Research Station both in the Zimbabwean tropical environments; and Ukulinga Research Station in the South African temperate environments.

Exposure of breeding material to contrasting environments ensures selection for broad range of abiotic and biotic stresses thereby enhancing chances of identifying adaptable germplasm (Ortiz et al., 2007). This observation warrants an investigation into the potential of using a "shuttle breeding" programme in facilitating identification of tropical germplasm that is adaptable to South African warm temperate environments. This will provide an alternative breeding strategy to current conventional breeding options being used by breeding companies targeting the South African market. An economic evaluation of the two programmes will be carried out to establish economic value of the alternative strategies for enhancing tropical germplasm to South African environments.

Economic evaluation

Genetic gain in maize germplasm improvement should be complemented with full consideration of cost effectiveness of the programme to ensure long term sustainability and profitability. Therefore, in this study a cost benefit analysis (CBA) will be carried out focusing on net present value (NPV) of overall cost-effectiveness of maize improvement alternative breeding strategies for enhancing adaptability of the tropical germplasm. According to Riley (2012), CBA is an important systematic process for calculating and comparing benefits and costs of a project for the purpose of: determining if a project is sound investment; and basis of comparing projects through comparison of the total expected cost of each option against the total expected benefits.

Summing up Rationale for Research Focus

Introgression of economically important specific traits from temperate germplasm is likely to enhance adaptability and productivity of tropical germplasm in the South African warm temperate environments. Utilization of a "shuttle breeding" programme will facilitate identification of adaptable introgressed lines relative to direct introduction of elite tropical parental lines and hybrids from breeding programmes operating from tropical environments. To build the base for future breeding progress it will be important to determine the level of genetic variation and genetic gain achieved through the incorporation of temperate germplasm in tropical elite inbred lines relative to their parental inbred lines and selection environments. An important objective of maize breeding programmes is to develop and use inbred lines that have good *per se* and *inter se* performance for target environments. Hence, introgressed lines will be assessed for their adaptability and productivity in South African warm temperate environments. Subsequently, economic and genetic value of a "shuttle breeding" programme will be evaluated relative to current conventional programmes for adapting tropical germplasm in the South African environments. Viability of any maize breeding programme must be justified by the release of productive products such as

inbred lines and hybrids that are cost effective. The cost of research must be minimized to ensure long term sustainability of the programme.

Research Objective

The aim of the study is to determine the genetic and economic value of a "shuttle breeding" programme for facilitating identification of adaptable tropical maize germplasm in the South African environments. The following specific objectives were pursued to:

1. determine effects of introgression and selection environments on performance and genetic variation of introgressed maize inbred lines
2. assess genetic gains for introgression of temperate germplasm into tropical elite maize inbred lines
3. assess yield stability and genotype adaptability of the single cross hybrids, and
4. determine the cost benefit analysis of conventional and "shuttle breeding" programmes.

Research Hypothesis

The following hypotheses were tested:
1. introgression of temperate germplasm in tropical elite inbred lines and selection environment does not alter genetic diversity and heterotic patterns of introgressed lines.
2. there is large genetic variation, heritability and positive association for grain yield and its components in the set of introgressed lines that can be explored in breeding programmes
3. there is large genetic gain and introgressed inbred lines performance *per se* that can be identified and exploited
4. High genetic gain and introgressed lines' *inter se* can be found in single cross hybrids that are adaptable to South African warm temperate environments

5. experimental single cross hybrids were comparable to commercial checks and adapted to South African temperate environments, and

6. "shuttle breeding" programme results in cost effective identification of adaptable tropical germplasm for the South African environments.

Thesis Outline

The thesis is structured as follows:

Introduction to thesis

Chapter 1: Literature Review.

Chapter 2: General Materials and Methods.

Chapter 3: Molecular characterization of maize introgressed lines bred in different environments.

Chapter 4: Genetic variation and Path Coefficient Analysis of introgressed inbred lines for economic traits.

Chapter 5: Assessment of genetic gains for introgression of temperate germplasm into tropical elite maize inbred lines: I. Performance *per se.*

Chapter 6: Assessment of genetic gains for introgression of temperate germplasm into tropical elite maize lines: II. Performance *inter se.*

Chapter 7: Stability assessment of single cross hybrids using GGE-biplot analysis

Chapter 8: An economic appraisal of conventional and "shuttle breeding" programmes: Implications for small and medium enterprises in the seed industry.

Chapter 9: Overview.

The thesis consists of nine chapters, each chapter is an independent potential manuscript for journal publication and therefore there may be overlaps and repetition of content and references with other chapters.

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1 Literature Review

1.1 Introduction

The chapter reviews literature on topics that are relevant to the study and provides a theoretical basis for the study. It seeks to give insight into assessing the potential effect of introgressing temperate germplasm into tropical elite inbred lines to enhance adaptability to South African warm temperate environments. "Shuttle" breeding and current conventional breeding programmes will be reviewed as alternative breeding strategies for enhancing adaptability of the introgressed lines and resultant hybrids in target environments. Genetic and economic gains of using the alternative breeding strategies in enhancing adaptability of tropical germplasm will also be reviewed.

1.2 Classification of maize germplasm

Maize (*Zea mays*, L) is ranked as a major cereal staple food crop grown in diverse environments. Its wide adaptability, economic and nutritional value has ranked it as the third most important cereal crop after wheat and rice. Wide genetic diversity exhibited in maize germplasm is perceived as the key to the regional and global success of maize as a source of food and industrial raw material. Xu et al. (2008) reports that the wide genetic diversity in maize has also resulted in it being defined relative to latitude and climatic region grown in, namely; tropical, sub-tropical, temperate and highland maize. The characteristic environmental conditions experienced by each maize class are depicted in Table 1.1. These classifications are not based precisely on geographical definitions but rather on agro-climatic criteria such as minimum and maximum temperatures and total rainfall during the growing season; altitude; and latitude. Maize hybrid oriented breeding programmes have further partitioned these maize germplasm classes into useable heterotic groups that ensure exploitation of hybrid vigour in hybrid development.

Table 1. 1: Maize class, environmental conditions and location

Maize class	Environmental conditions	Location
Tropical	-high mean temp around 28 °C during growing season. -high mean max temp around 32 °C -high mean min temp around 22 °C -high day-time and night temperatures occur throughout the growing season	-Between tropic of Cancer 23° N and tropic of Capricorn 23° S at elevations below 1 000 m a.s.l. - 60 countries of Asia, Latin America and Africa.
Subtropical	-cooler mean temp around 25 °C -high mean day-time temp similar to tropical -occur at high elevations hence lower mean night temp -longer growing seasons than tropical	- Between tropic of Cancer 23° N and tropic of Capricorn 23° S at elevations above 1 000 m a.s.l -Southern Africa, Southern Brazil, Mexico and Central America, Egypt, South Asia and China.
Temperate	-mean temperatures of about 20 °C during growing season. -mean max temp during the day around 24 °C -mean min temp during the night around 14 °C -frost is a major threat -receives more hours of solar radiation per day than latitudes closer to the equator.	- Above 23 ° S latitude N and S to Arctic and Antarctic respectively. -China, Argentina, Australia, South Africa, North and South America, Middle East and Europe
Highland	-mean daily temp 16 to 18 °C -mean max temp during the day around 25 °C -mean min temp during the night around 8 °C	-1 800 and 3 000 m a.s.l in tropical latitudes 23° North or South and between 1 600 and 2 700 m a.s.l in subtropical 23° to 34° North and South -Eastern and Southern Africa, Mexico, Central America, Pakistan, Nepal, India, China and South America.

(Adjusted after Dowswell et al. (1996) and Xu et al (2009)) *Abbreviations*: min, minimum; max, maximum; temp, temperature; m.a.s.l, meters above sea level

1.2.1 Maize heterotic groups

Effective utilisation of maize inbred lines in hybrid maize development is mainly dependent on establishment and maintenance of heterotic groups within a breeding population. Heterotic groups are defined as "a set of inbred-line populations with rich genetic background, similar main characteristics trends and strong general combining ability". Knowledge of heterotic groups ensures that maximum heterosis is achieved through hybrid combinations created between inbred lines from genetically divergent clusters. Heterosis or hybrid vigour is defined as superior performance of hybrids compared to their parental inbred lines (Shull, 1908). However, extensive field trials carried out in breeding programmes to assess heterosis are often time consuming

and costly. Hence, breeding programmes depend on the concept of heterotic groups, where the breeding material is assigned to genetically divergent heterotic pools (Thiemann et al., 2010).

Breeding programmes exploit the heterotic groups and patterns through development of inbred lines within the same group and maximising on heterosis or hybrid vigour by crossing inbred lines categorised in dissimilar groups. Derera (2005), reports that the best hybrid vigour or heterotic responses are obtainable when crosses are made between parents originating from genetically different populations. Therefore, there is need to establish the probable new heterotic groups to ensure exploitation of heterotic patterns and effects of selection environment that might have been generated in the population for enhanced productivity.

Table 1. 2: Main heterotic groups of maize inbred lines used in Eastern and Southern Africa

Heterotic Group	Population of derivation	Examples of Public lines	Reference
SC	Southern Cross	SC5522	Mickelson et al. (2001)
N3	Salisbury White	N3-2-3-3	Mickelson et al. (2001)
K	K64R/M162W	K64R/M162W	Mickelson et al. (2001)
P	Natal Potchefstroom Pearl Elite Selection (NPPES)	NAW5867	Gevers and Whythe (1987) Olver (1998)
I	NYHT/TY	A26, I137TN	Gevers and Whythe (1987)
M	21A2.Jellicorse	M37W	Gevers and Whythe (1987)
F	F2934/Teko Yellow	F2834T	Gevers and Whythe (1987)
CIMMYT-A	Tuxpeno, Kitale, BSSS, N3 (More dent)	CML442, CML202	CIMMYT, 2001
CIMMYT-B	ETO, Ecuador 573, Lancaster, SC (More flint type)		

Source: Derera 2005.

Several studies in maize breeding programmes have reported on heterotic patterns used globally. In Eastern and Southern Africa, there are at least nine main heterotic groups (Table 1.2). K64R is a direct import from the USA, with SC, N3, and NPPES being derived from varieties imported from the USA (Mickelson et al., 2001). Hybrids from national breeding programmes in Eastern and Southern Africa have SC, N3 and

K64R derived lines as key components (Olver, 1998). Early maturing hybrids in Zimbabwe are mainly constituted from K64R lines and their derivatives (Olver, 1998). Derera (2005), reports that CIMMYT has two classes A and B; with the classification being broader than the regional categorisation. A number of methods are used in establishing heterotic groups and patterns in maize breeding programmes namely: pedigree analysis, quantitative genetic analysis and molecular markers.

Pedigree analysis classifies inbred lines based on pedigree records which are generated from traditional phenotypic selection methods and geography (Zhang et al., 2002; Cheng-Lei et al., 2010). However, pedigree analysis is limited by reliability of pedigree records and morphological traits involved. Morphological traits have a limitation due to difficult to detect alleles among germplasm and environment influence resulting in reduced precision. Quantitative genetic analysis technique is based on specific combining ability data generated from mating designs such as diallel to classify the inbred lines into heterotic groups (Zhang et al., 2002). Reliability for specific combining ability depends upon the quantity of inbred lines used as parents and the genetic base of the entries. Hence the use of new powerful and effective methods that are independent from environmental influence and can be detected at any stage of plant development, namely the use of molecular genetic markers has been used in recent years.

1.3   Molecular Markers

Molecular markers provide a precise tool for estimating genetic diversity of a given population. A number of molecular markers are currently available for use in diversity studies. These include Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Rapid Amplified Fragment Polymorphism (RAPD), Simple Sequence Repeats (SSRs), Single Nucleotide Polymorphism (SNP) and Inter-Simple Sequence Repeats (ISSRs) (Table 1.3). However, this variety of available markers can possibly pose challenges for researchers as each marker has its merits and demerits (Table 1.3). Therefore, the choice of which marker to use will often be project specific in order to ensure efficiency.

The simple sequence markers (SSR) are currently the most widely used molecular markers. They are highly polymorphic, even between closely related inbred lines, require low amounts of DNA, can easily be automated for high throughput screening, and are highly transferable between populations (Semagn et al., 2006). Simple sequence repeat (SSR) markers have been suggested as the markers of choice in several studies (Lu et al., 2009; Cheng-Lai et al., 2010; Prasanna et al., 2012) for illustrating the importance of genetic diversity of tropical germplasm in maize breeding programmes. These studies defined tropical germplasm as the most genetically diverse source of germplasm with favourable alleles for yield incorporation and resistance or tolerance for biotic and abiotic stresses. Genetic diversity for high yield, low grain moisture content at harvest and good stand ability using SSR makers in temperate germplasm has also been reported (Lu et al., 2009; Xu et al., 2009; Prasanna et al., 2004). However, there has been no genetic diversity analyses reported for temperate germplasm introgressed into tropical elite inbred lines in Southern Africa, in particular South Africa. Availability of such information would be key to the utilisation of tropical elite germplasm that is adaptable and productive in South African warm temperate environments. In this study SSR markers were used to establish heterotic groups and patterns in the new inbred lines; while SNP markers were used to establish level of homozygosity in the inbred lines.

1.4   Genetic diversity

Exploitation of the knowledge of genetic diversity in a breeding population forms the basis of breeding gains in trait improvement. Genetic diversity is defined as a measure of phenotypic and genotypic variance in field evaluation (Yoshida and Yoshida, 2004). According to Lu et al. (2009), genetic diversity studies give a detailed knowledge of the relation between maize inbred lines. This is important, not only for parental selection, but also for efficient genetic analysis and effective exploitation in breeding programmes. Commercial maize hybrid varieties are developed through prediction of performance of intergroup crosses. Crosses are made from inbred lines from opposite and complementary heterotic groups in order to maximize heterozygosity in the hybrid thus hybrid performance (Lu et al., 2009). Tropical and temperate germplasm defined in Table 1.1 possess wide genetic diversity that can be exploited in maize breeding programmes. Therefore, it is important to explore genetic diversity in tropical and temperate germplasm to ensure development of maize hybrid varieties that have high agronomic performance across target environments.

1.4.1   Opportunities in maize germplasm improvement

Genetic diversity in maize across temperate and tropical germplasm offers possible opportunities for genetic enhancement and increase in maize yield production. With the current knowledge on genetic diversity of maize germplasm, breeding programmes can be more efficient and effective in enhancing genetic gain in hybrid maize grain yield (Laborda et al., 2005). According to Wende et al. (2012), genetic gain during selection is dependent on the presence of genetic diversity for the desired economic traits. Both temperate and tropical germplasm possess variable favourable alleles of important traits which can be exploited for maize germplasm improvement.

Table 1. 3: Comparison of the five most extensively used DNA markers in plants

	RFLP	SNP	Microsatellite	RAPD	AFLP	ISSR
Genomic abundance	high	high	medium	very high	very high	Medium
Part of genome surveyed	low copy coding regions	whole genome	whole genome	whole genome	whole genome	whole genome
DNA required	high	low	low	low	medium	low
Type of polymorphism	single base changes, insertion, deletion	single base change	changes in length of repeats	single base changes, insertion, deletion	single base changes, insertion, deletion	single base changes, insertion, deletion
Level of polymorphism[a]	medium	high	high	high	very high	high
Effective multiplex ratio[b]	low	high	medium	medium	high	medium
Marker index c	low	high	medium	medium	high	medium
Inheritance	codominant	codominant	codominant	dominant	dominant	dominant
Detection of alleles	yes	yes	yes	no	no	no
Ease of use	Labour intensive	easy	easy	easy	difficult initially	easy
Automation	low	high	high	medium	medium	medium
Reproducibility (reliability)	high	high	high	intermediate	high	medium to high
Type of probes/primers	low copy genomic DNA or cDNA	specific 18-23 bp	specific repeat DNA sequence	usually 10 bp random nucleotides	specific sequence	specific repeat DNA sequence
Cloning and/or sequencing	yes	yes	yes	no	no	no
Radioactive detection	usually yes	no	no	no	yes/no	no
Development/start-up costs	high	high	high	low	medium	medium
Utility for genetic mapping	species specific	species specific	species specific	cross specific	cross specific	cross specific
Proprietary rights status	No	No (some are licensed)	No (some are licensed)	licensed	licensed	no

RFLP-restriction fragment length polymorphisms; Microsatellites-simple sequence repeat markers; SNP-single nucleotide polymorphism; RAPD-random amplified polymorphic DNA; AFLP-amplified fragment length polymorphism; ISSR-inter simple sequence repeats.

a Level of polymorphism (average heterozygosity) is an average of the probability that two alleles taken at random can be distinguished

b Effective multiplex ratio is the number of polymorphic loci analysed per experiment in the germplasm tested.

c Marker index is the product of the average expected heterozygosity and the effective multiplex ratio.

Source: adjusted after Semagn et al., 2006)

1.4.2 Tropical germplasm

Tropical maize germplasm represents a diverse source of germplasm for desired economic traits in breeding programmes. According to Nelson and Goodman (2008), for many years maize breeders have advocated breeding with tropical germplasm (Vasic et al. 2006; Nelson et al. 2006), which is the logical source of added genetic diversity. Tropical maize germplasm has high yield potential and tolerance to diseases and abiotic stresses; resistance to a wide range of pests; drought and heat stress relative to temperate germplasm (Tarter et al., 2004; Betran et al., 2006). Successful utilisation of tropical germplasm as a source of exotic germplasm in temperate breeding programmes in America, China and Europe is well documented (Gracen, 1986; Duvick, 2005; Xing-kui et al., 2013). In contrast, there is very limited information describing its potential use in South African warm temperate environments. In addition, the limited information currently available is mostly verbal communication, which may be subjective. There is a need to generate such quantitative information for South African warm temperate environments. This will allow breeding programmes to exploit the potential utilisation of tropical germplasm in breeding for adaptability to South African warm temperate environments.

The current lack of information has led to a situation whereby germplasm is being underutilised. According to Nelson and Goodman (2006), breeders have limited information on which to base parental choices. In addition, effective utilisation of unadapted tropical germplasm in temperate environments is expensive and laborious. A large breeding effort is required to successfully extract these traits into temperate germplasm. When directly introduced into temperate environments, tropical germplasm is characterised by lack of adaptability, namely: excessive rank growth; late flowering; excessive lodging; high grain moisture content at harvest and low harvest index (Abadassi and Herve, 2000; Uhr and Goodman, 1995). Hence, there is need to improve on the desired economic traits to enhance adaptability of the tropical germplasm in warm temperate environments.

1.4.3 Temperate germplasm

Narrow genetic diversity in temperate germplasm has been reported in a number of studies. Despite its narrow genetic base, breeding programmes from temperate environments in USA (Tarter et al., 2004; Duvick, 2005), France (Dowswell et al., 1996), China (Xing-kui et al., 2013), Argentina (Luque et al., 2006) and South Africa (Goodman, 1999) dominate global production. Temperate maize germplasm is characterised by desirable attributes such as: early maturity; good plant stand ability; upright or erectophile architecture which are adapted to high planting density; small tassel size; productive tillering; ear prolificacy; and low grain moisture content at harvest (Gracen, 1986; Duvick, 2005; Hai-ming et al., 2006). However, the narrow genetic base of temperate germplasm renders it vulnerable to pests or pathogens and hence a loss of stability of maize yields (Gracen, 1986).

The advantages of tropical germplasm and the limitations of temperate germplasm highlight areas of possible complementarity. Therefore, there is need to exploit complementary traits available in temperate germplasm in breeding tropical germplasm for adaptability to the South African warm temperate environments. This might provide, not only increase in genetic diversity, but also enhanced adaptability of tropical germplasm to South African warm temperate environments. Economic traits that would be expected to benefit from such introgression include plant stand ability, early physiological maturity and high grain yield potential. However, to ensure realisation of these desired improvements, there is also a need to have an effective breeding strategy.

1.5 Breeding strategy

Breeding strategy can be defined as the formulation of the theory of selection, genetic and quantitative genetics as well as further systematic application by a plant breeder in providing a framework for the sustained genetic improvement of yield and agronomic traits (Messina et al., 2011). Two such strategies that have received

widespread practical application in breeding for adaptability to target environment are incorporation and introgression of genes from exotic germplasm.

1.5.1 Incorporation of genes from exotic germplasm

Betran et al. (2006) defines incorporation of exotic genes as utilisation of foreign germplasm in adapted maize germplasm. This is achieved through direct selection of inbred lines from exotic germplasm without hybridisation with adapted germplasm or from populations with high percentage of exotic germplasm (> 75%). It is reported to be a long-term breeding programme for unadapted exotic germplasm to generate a locally adapted genetic base that can be used as complementary parental lines (Simmonds, 1993). According to Goodman et al. (2000) it is a potentially powerful approach in increasing genetic diversity as it is a population-oriented approach rather than a gene or trait based approach. However, it has its challenges, namely: long-term project to achieve substantial progress; there is no guarantee that the phenotypes of exotic material will provide any desirable guide to breeding worth; and developed population has to complement the adapted maize germplasm. Despite its challenges, incorporation of exotic germplasm has been successfully used in the USA's temperate breeding programmes to develop inbred lines (Simmonds, 1993; Betran et al., 2006).

1.5.2 Introgression of genes from exotic germplasm

Introgression of exotic genes into adapted germplasm to enhance trait improvement has been reported as a short-term project to attain progress for desired economic traits. Simmonds (1993) defines introgression as focus on the use of small number of entries of exotic germplasm explicitly tested for desired traits introduced by crossing into adapted germplasm, with the appropriate selection over generations. It is an approach which is suitable for short-term breeding projects which introduces a few desirable genes and does very little in terms of improving the genetic base of the adapted maize germplasm (Simmonds, 1993; Goodman et al., 2000). Contrary to Simmonds (1993), Goodman et al. (2000) and Betran et al. (2006), a number of

studies (Abadassi and Herve, 2000; Lewis and Goodman 2003; Tarter et al., 2004; Nelson et al., 2006; Nelson and Goodman, 2008) have reported not only the potential of exotic maize germplasm in improving adaptability and yield but also increasing genetic diversity. Breeding programmes in China (Hai-ming et al., 2006); Argentina (Luque et al., 2006), Brazil (Paterniani, 1989), USA (Duvick, 2005; Hai-ming et al., 2006), all temperate environments, have successfully used tropical and temperate exotic germplasm to enhance genetic gains for grain yield thus increasing maize production. Despite the gains achieved in these programmes, there is limited or poor documentation on the use of temperate germplasm in improving adaptability of tropical germplasm to the South African warm temperate environments. There remains a need to exploit introgression of temperate germplasm into tropical elite maize inbred lines to enhance adaptability and productivity in the South African warm temperate environments. To ensure enhanced adaptability of the tropical germplasm, focus should be on selecting of desired economic traits.

1.6   Desired economic traits

1.6.1 Ear prolificacy

Ear prolificacy in maize is defined as the ability to produce multiple cobs per plant and has high positive correlation with yield. In South African warm temperate maize production, ear prolificacy is a trait linked to adaptability of a cultivar and can be used to good advantage where lower plant populations are required (du Plessis, 2003). According to Brathwaite and Brathwaite (2002) and Kesomkeaw et al. (2009), a number of studies have reported genetic diversity and high heritability for prolificacy in temperate germplasm; however there are very few reports on tropical germplasm. In addition, previous research by Varga et al. (2004) and Svecˇnjak et al. (2006) reported greater efficiency in resource utilisation under stress in prolific maize hybrids. This suggests that the trait can be targeted in breeding maize for adaptability. Therefore, introgression of the ear prolificacy trait in tropical germplasm may provide a major possible breeding strategy in addressing adaptability of tropical germplasm to South African warm temperate environments. In addition, poor plant stand ability has

often been cited as a major limiting factor restricting the use of prolific hybrids (Varga et al. 2004). As such, there is need to focus on selecting introgressed lines and hybrids that are both prolific and with good stand ability.

1.6.2   Plant stand ability

Tropical germplasm generally has a weak root and stalk system which increases its susceptibility to lodging. According to Nelson and Goodman (2008), tropical germplasm is characterised by excessive rank growth which results in increased root and stalk lodging when directly introduced into temperate environments. Temperate germplasm, on the other hand, provides a good source of genes for good stand ability in maize breeding. Duvick (2005) and Gracen (1986) report that temperate germplasm, in particular stiff stalk synthetic (BSSS) genetic background and its derivatives; have high genetic diversity and heritability for resistance to root and stalk lodging. Olver (1998), reports on the potential of utilising temperate germplasm in improving tropical germplasm plant stand ability. Hypothetically, this would provide potential germplasm for exploitation in improving stand ability of tropical germplasm in South African warm temperate environments, thus increasing adaptability.

1.6.3   Grain moisture content at harvest

A number of studies have reported genetic variation for grain moisture content at harvest in tropical and temperate maize germplasm. Grain moisture content is an important physiological process influenced by weather conditions and genotypes (Filipenco et al., 2013). In maize production, it is an economically important trait that can increase growers' production costs in relation to artificial grain drying and losses due to delayed harvesting (lodging, bird and insect damage, and ear rot diseases) (Yan, 2011; Filipenco et al., 2013). Tarter et al. (2004) and Goodman (2005) report that tropical germplasm, when directly introduced in temperate environments, has high grain moisture content at harvest. It is therefore prudent to introgress temperate germplasm to lower the grain moisture content of tropical germplasm at harvest and hence enhance adaptability.

1.6.4 High grain yield

Breeders and farmers prefer maize hybrid varieties that are tolerant to abiotic and biotic stresses but most importantly a high yielding variety. Uhr and Goodman (1995) and Luque et al. (2006) report that tropical germplasm is a valuable source of exotic genes to increase yield (average of about 1.0 t ha^{-1} at a cost of 1.6% grain moisture and 8% lower stand ability) in breeding programmes. In addition, Duvick (2005) also reports that tropical germplasm has had an estimated average genetic gain of 145 kg ha^{-1} year^{-1} between the 1979-1998 period, while temperate germplasm has had an estimated genetic gain of 66 kg ha^{-1} year^{-1} between the 1930-1991 period. This indicates that there may be potential to get increased genetic gain for grain yield through the utilisation of tropical germplasm. It can be hypothesised that introgressing temperate germplasm into high yielding tropical elite inbred lines while eliminating undesirable traits will enhance genetic gain in yield and adaptability of tropical germplasm to South African temperate environments.

1.7   Genetic gain and productivity of hybrids

Establishment of breeding progress achieved in improving desired economic traits is important in ensuring effective progress during selection. Tropical and temperate breeding programmes have noted breeding progress as reflected in increased genetic gain and productivity in maize hybrid varieties across target environments. Singh and Chaudhary (2004) define genetic gain as genetic advance attained between means of selected progenies and the base population under a specific selection pressure. Genetic gain can be estimated as: realised genetic gain-actual achievable gain in a breeding programme (Weng et al., 2008); or predicted gain-expected gain in breeding programmes (Callister et al., 2013). Gapare (2000) reports that for genetic gain to be achieved there must be genetic variation present within the population and also a degree of heritability for the desired trait. Estimation of genetic gain is essential in determining the cost effectiveness of the breeding strategy being implemented and the productivity of improved germplasm relative to the unimproved. In maize breeding

the primary objective is to develop hybrid varieties that have high yield potential and productivity across target environments.

1.7.1 Genotype by environment interactions

Variable growing environments pose a major challenge due to significance of genotype-by-environment interaction. Therefore, there is need to recommend consistent and productive cultivars and areas of specific adaptation through evaluation of multi-locational trials. A number of techniques have been utilised for analyses of adaptability and stability in target environments namely: analysis of variance (ANOVA); linear regression analysis; non parametric tests; multivariate analysis-principal component analysis, additive main effects and multiplicative interaction (AMMI), genotype, genotype x environment (GGE) biplot and cluster analysis (Balestre et al., 2009). Analysis of variance technique is an additive model that measures the main effects and determines if genotype-by-environment interaction is a significant source of variation, but does not provide insight into the genotypes or environments that give rise to the interaction (Samonte et al. 2005).

Linear regression is a technique that was developed by Finlay and Wilkinson (1963) and used for analysis of adaptation of genotypes in target environments. Complex interactions are simplified into linear responses when genotypes exhibit a linear response to the target environments (Eberhart and Russell, 1965). However, linear regression analysis is poor at handling outliers (genotype or environment) and incomplete data sets. Non–parametric techniques measure stability of genotypes based on ranks of genotypes in different environments. It provides useful alternative parametric measures which are not based on absolute values. Unlike linear regression analysis, statistics generated are not influenced by addition or deletion of genotypes or grouping of material into sets (Huehn, 1990). Cluster analysis technique is a stability based method that provides a way of validating the quality of the data and revealing of patterns according to prior knowledge (Ben-Hur et al., 2001).

Principle component analysis (PCA) is the most frequently used multivariate technique, which is a generalisation of linear regression analysis technique (Crossa, 1990). Furthermore, PCA can effectively reduce the structure of a two way genotype-by-environment interaction data matrix of genotypes (G) points in environments (E) dimensions in subspace of fewer dimensions. Under multivariate techniques, AMMI analysis and a modification of the conventional AMMI analysis GGE-biplot analysis – are two methods that have been consistently used in a number of studies. According to Balestre el al. (2009), AMMI analysis interprets the effects of genotypes and environments as additive and GE interaction as multiplicative, by principle component analysis. While the GGE-biplot analysis groups the genotype effect which is an additive effect in AMMI analysis together with the GE interaction, multiplicative effect, and analyses these effects by principal components (Kaya et al., 2006). With regards to this study, GGE-biplot analysis technique will be used to evaluate the adaptation of grain yield and its components of the improved tropical single cross hybrids in South African target environments.

The issues raised by the genotype–environment analyses, allude to the need to develop hybrid cultivars that are widely adaptable and productive. Development of such hybrids should be complemented with cost effective and productive implementation of breeding programmes. In this regard, it is important to estimate genetic gain attained through introgressing temperate germplasm in tropical germplasm to enhance adaptability and productivity under South African warm temperate environments. In addition, to enhance adaptability and productivity an effective selection approach has to be applied during breeding.

1.8   Enhancing adaptability through a "Shuttle breeding" programme

Ortiz et al. (2007) and Khush (2001), define a "shuttle breeding" programme as a breeding approach in which segregating populations are screened in two or more contrasting environments and then tested under near optimum conditions for yield evaluation. Multiple testing of the screened lines and hybrids is then carried out at sites that represent defined mega environments so as to identify germplasm that is

adaptable. During this process, pyramiding of a sizeable amount of numerous genes adaptable within each distinct environment is achieved and germplasm is obtained that is highly productive in several environments. According to Oritz et al. (2007), such an approach has yielded the success collectively known as the Green Revolution. The International Maize and Wheat Improvement Centre's (CIMMYT) wheat programme produced wide adaptation, durable rust and septoria resistance and appropriate use of genetic variation to enhance yield gains of subsequently produced lines; through the use of a "shuttle breeding" programme.

Despite the success that has been witnessed in the CIMMYT wheat breeding programme using the "shuttle breeding" programme, there has been limited application of this programme in maize breeding. An exception is Troyer (1996), who reported that the Pioneer hybrid maize breeding programme and Green Revolution mutually benefited from each other's success. This indicates that the "shuttle breeding" programme has yielded more widely-adapted maize hybrid varieties that have been grown in the USA's temperate environments. However, there has been limited or no information on the use of a "shuttle breeding" programme using tropical germplasm in breeding for adaptability to the South African warm temperate environments. The success of the 'shuttle breeding' programme suggests that it could be exploited using tropical germplasm selected at distinct selection environments in breeding for wide adaptability and productivity for South African warm temperate environments. The focus would be on selecting for desired traits that enhance adaptability in the temperate environments, namely; ear prolificacy, plant stand ability, early physiological maturity and high grain yield. However, South Africa currently relies on conventional breeding strategies. A shift from these to the "shuttle breeding" would have to be justified in terms of not only developing new adapted hybrids, but also on cost.

1.8.1   Economic analysis of the alternative breeding strategies

Current conventional breeding strategies being implemented by breeding programmes operating outside South Africa have failed to address the challenge of lack of adaptability that characterises tropical germplasm under temperate environments. Their approach has centred on breeding and selection of lines and hybrids in tropical environments and the resultant products are then directly introduced into South Africa's warm temperate environments. Diverse climatic differences between the tropical selection environments and the temperate evaluating environments render the germplasm unadaptable. Therefore, there is a need to compare the effectiveness of a "shuttle breeding" programme to enhance adaption of introgression inbred lines to South African warm temperate environments relative to conventional breeding programmes that are normally used. Such a comparison should be accompanied by a cost benefit analysis (CBA) with emphasis on net present value (NPV) to ensure long term sustainability and profitability of the project. There is a dearth of literature describing such an economic comparison. If such a comparison were to be done, it would set a precedent that can be applied and/or adopted by breeding programmes operating in tropical environments targeting South African warm temperate environments.

1.9   Summary of literature review

Maize remains an important crop in Sub-Saharan Africa. This has necessitated several breeding programmes by local and international breeding companies. These breeding programmes have opted for different approaches: breeding companies operating outside South Africa (tropical breeding programmes) opting to directly introduce their elite germplasm into the predominantly warm temperate South African environments. The review highlighted that breeding programmes in tropical environments should consider opportunities in breeding strategies and genetic diversity of tropical and temperate germplasm in breeding for adaptability to South African warm temperate environments. In addition, the literature indicates that there is a possibility of introgressing genes from temperate germplasm into tropical

germplasm to enhance adaptability to South African warm temperate environments. The success of any breeding programme is dependent on the effectiveness of the breeding strategy employed to select and adapt for desired traits. Conventional breeding programmes currently being utilised by seed companies operating outside South Africa have so far resulted in limited success. Hence, there is need to consider switching to a "shuttle breeding" programme. The "shuttle breeding" programme provides a possible viable option for enhancing wide adaptability of introgressed lines during selection and advancement. There is no literature comparing conventional and shuttle breeding programmes in relation to the economic and genetic benefit in breeding. However, efficient breeding programmes have to be cost effective (low operation cost) but at the same time produce adapted and productive maize hybrid varieties. Hence, it will be very prudent to understand the genetic gains in terms of productive inbreds and hybrids. The economic gains in terms of cost effectiveness of using alternative breeding programmes in enhancing adaptability of tropical germplasm to South African warm temperate environments also requires attention.

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2   General Materials and Methods

2.1   Germplasm

2.1.1   Introgression Procedure

A single common donor maize parental inbred line (08CED6_7_B) from South Africa was used to introgress genes from temperate germplasm into 12 elite tropical inbred lines from Zimbabwe through pedigree crosses in 2008 in South Africa. Tropical maize population lines used were a representative of the major tropical heterotic groups mainly N_3 (derived from Salisbury white), SC (Southern Cross which was derived from an open pollinated population grown by Mr South in Zimbabwe) and P (derived from the open pollinated variety (OPV) Potchefstroom Pearl). The temperate maize population was one of the major temperate heterotic groups used in South Africa (TAB population). Hand crossings were made between the tropical and temperate populations to generate F_1 hybrid seed. Due to challenges in flowering synchronization (nicking) and seed availability a total of eight populations were generated for advancement and selection at F_2 generation.

2.1.2   Advancement of breeding populations

Each population was independently advanced from F_3-F_6 generation though selfing and selection of adapted segregants in a "shuttle" and conventional pedigree breeding programmes as described below. Advancing of each population through selfing increased level of homozygosity of the population resulting in increased uniformity within each family. As homozygosity increased, traits that were caused by recessive alleles were exposed during inbreeding. The negative effects of inbreeding are referred to as inbreeding depression which resulted in reduction in height reduction, loss in vigour and poor pollen production in some maize inbred lines with each generation of inbreeding cycle.

2.1.3 "Shuttle breeding" programme

In the "shuttle breeding", segregants from each population were independently advanced at three distinct environments namely Rattray Anorld Research Station and Kadoma Research Centre which are both tropical environments in Zimbabwe, and Ukulinga Research Station in South Africa representing warm temperate environment. Temperate donor inbred line was crossed to tropical elite inbred lines in South African warm temperate environment; resultant F_1 seed was advance to F_2 generation where major selection was carried out. Subsequent F_3 generation was split into three identical sets for advancement in parallel programmes at the three sites. Selection and advancement of material was carried out at each environment relative to adaptability to stress exposure at each selection environment as indicated in Table 2.4. The resultant genotypes were introgressed lines referring to recombinant tropical inbred lines that combined tropical with temperate germplasm. Advancement of the progenies was carried out up to F_6 generation within each environment. Pedigree selection emphasized early flowering (anthesis and silking days), low grain moisture content at harvest; good plant standing ability; ear prolificacy and high grain yield potential. However, the most important trait in the selection index was the ear prolificacy. The schematic flow of a "shuttle breeding programme is shown in Figure 2.1.

2.1.4 Conventional pedigree breeding programmes

Parallel to the "shuttle breeding" programme, two schemes for conventional breeding were carried out as shown in Figure 2.2. Two conventional breeding programmes were established in Zimbabwe at Rattray Anorld Research (tropical environment) and in South Africa at Ukulinga Research Station (temperate environment). Inbred lines generated in each programme were used as representative lines for comparison in the study.
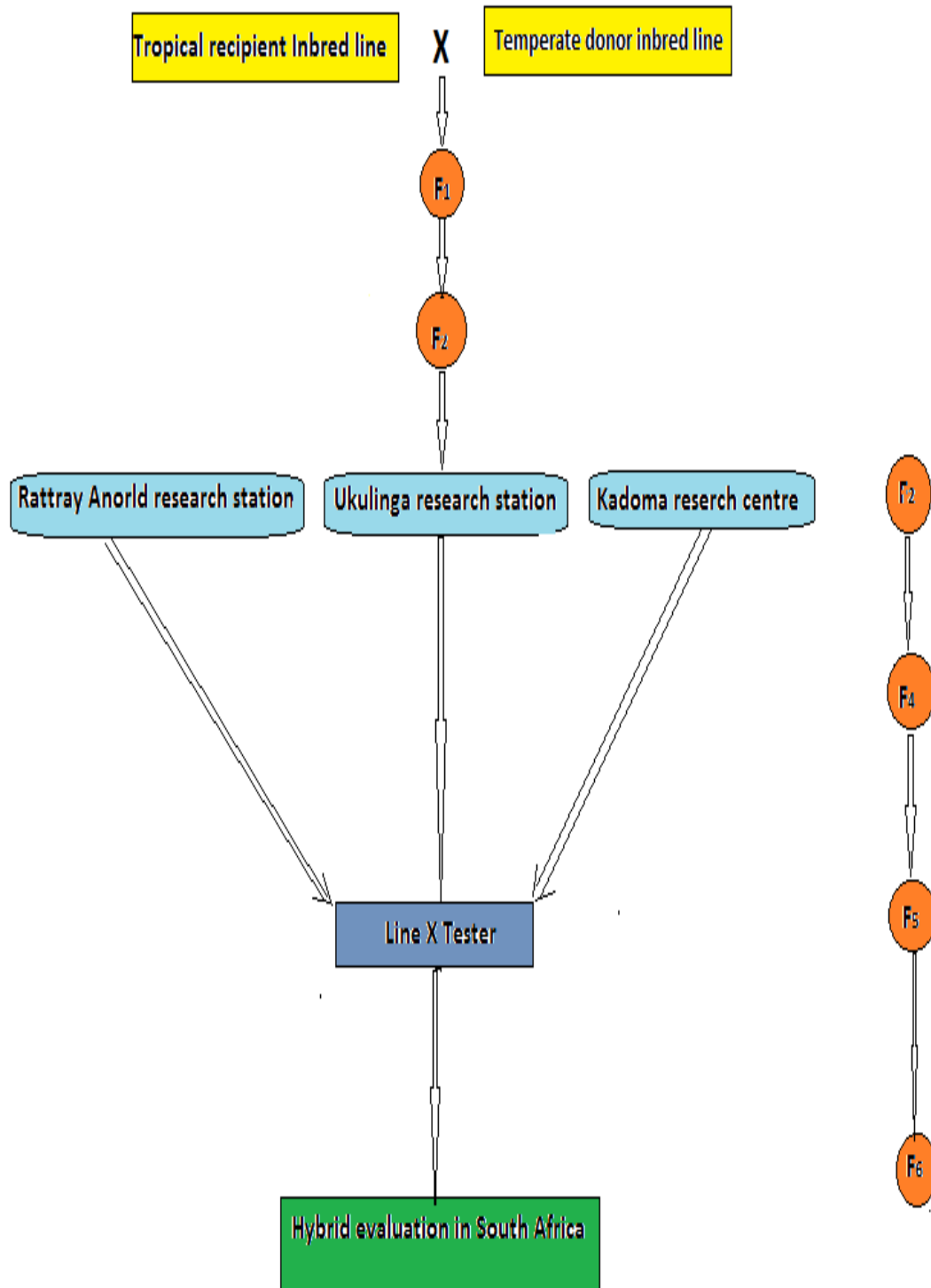
Figure 2. 1: Schematic flow of a shuttle breeding programme
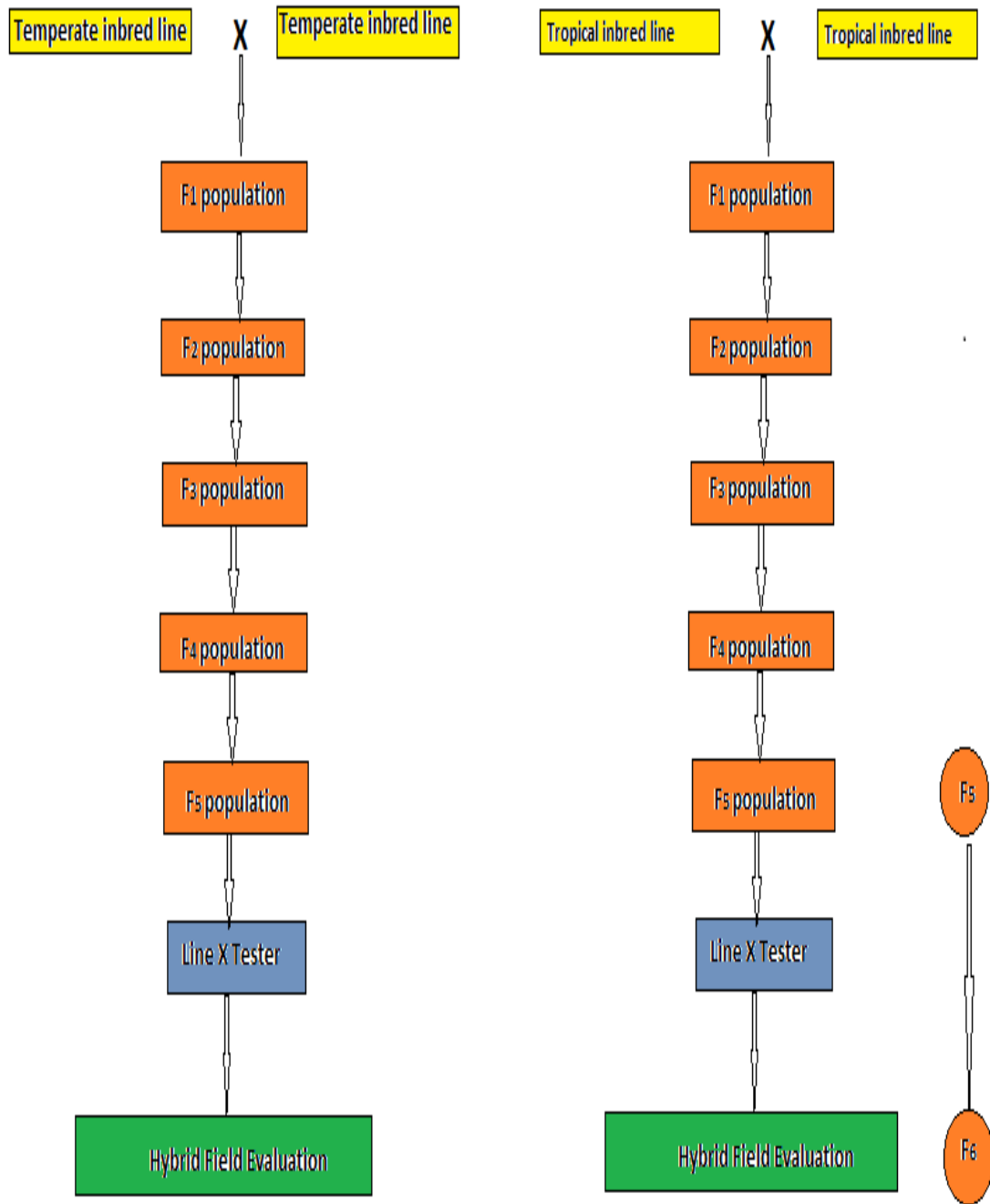
(Hybrid Evaluation carried out in South Africa for both programs)

Conventional breeding in South Africa   Conventional breeding in Zimbabwe

Figure 2. 2: Schematic flow of parallel conventional breeding programmes carried out in South Africa and Zimbabwe

2.1.5   Test crossing study population

Test-crossing of introgressed lines was carried out at F_5 generation for all breeding programmes using a Line X Tester mating design. In the "shuttle breeding" programme four tropical background testers were used that were designated as: T1, T2 and T3 which are in the N heterotic group; and T4, which is in P heterotic group. These were crossed to 50 selected introgressed lines from each of the three selection environments to produce a total of 600 single cross hybrids.

Under the conventional breeding programmes, adapted tropical based testers (T1, T2 and T4) and temperate based testers (T9 and T10) were used to produce single cross hybrids in each respective conventional breeding scheme and the resultant single cross hybrids were also evaluated in hybrid evaluation trials, respectively.

2.1.6   Fixing of selected lines

Advancement of introgressed lines from F_5 –F_6 generation was carried out in summer 2012 at two different locations, Ukulinga Research Station and Kadoma Research Centre described in Table 2.4.   Resultant introgressed lines from the "shuttle breeding" programme represent tropical maize inbred lines introgressed with genes from temperate germplasm. The two conventional breeding schemes produced tropical inbred lines that were adaptable to Zimbabwean tropical environments and temperate inbred lines that were adaptable to South African warm temperate environments. Random sample of: the introgressed lines that were bred from the "shuttle breeding" programme; tropical inbred lines bred from the conventional pedigree breeding programme in Zimbabwe; temperate inbred lines bred from South African conventional pedigree breeding programme; and elite tropical lines representing heterotic grouping SC (Southern Cross), N3 (Salisbury white), K64r and P (Natal Potchefstroom Pearl Elite Selection) were used for field evaluation in trials. The levels of fixation (homozygosity of the lines is presented in Table 2.1 to 2.3.

Table 2. 1: Description of maize inbred lines used in the study

Entry	Code	Breeding Environment	Germplasm background	Homozygosity
1	KRC_1	KRC-Zimbabwe	Introgressed lines	0.90
2	KRC_2	KRC-Zimbabwe	Introgressed lines	0.92
3	KRC_4	KRC-Zimbabwe	Introgressed lines	0.94
4	KRC_5	KRC-Zimbabwe	Introgressed lines	0.68
5	KRC_6	KRC-Zimbabwe	Introgressed lines	0.99
6	KRC_7	KRC-Zimbabwe	Introgressed lines	0.63
7	KRC_8	KRC-Zimbabwe	Introgressed lines	0.74
8	KRC_9	KRC-Zimbabwe	Introgressed lines	0.80
9	KRC_11	KRC-Zimbabwe	Introgressed lines	1.00
10	KRC_22	KRC-Zimbabwe	Introgressed lines	0.98
11	KRC_23	KRC-Zimbabwe	Introgressed lines	0.56
12	KRC_24	KRC-Zimbabwe	Introgressed lines	1.00
13	KRC_25	KRC-Zimbabwe	Introgressed lines	1.00
14	KRC_27	KRC-Zimbabwe	Introgressed lines	0.98
15	KRC_28	KRC-Zimbabwe	Introgressed lines	0.99
16	KRC_29	KRC-Zimbabwe	Introgressed lines	1.00
17	KRC_30	KRC-Zimbabwe	Introgressed lines	0.95
18	KRC_31	KRC-Zimbabwe	Introgressed lines	0.95
19	KRC_33	KRC-Zimbabwe	Introgressed lines	1.00
20	KRC_34	KRC-Zimbabwe	Introgressed lines	1.00
21	KRC_35	KRC-Zimbabwe	Introgressed lines	0.99
22	KRC_38	KRC-Zimbabwe	Introgressed lines	0.85
23	KRC_39	KRC-Zimbabwe	Introgressed lines	0.96
24	KRC_41	KRC-Zimbabwe	Introgressed lines	0.71
25	KRC_43	KRC-Zimbabwe	Introgressed lines	0.69
26	RARS_1	RARS-Zimbabwe	Introgressed lines	0.66
27	RARS_2	RARS-Zimbabwe	Introgressed lines	0.94
28	RARS_3	RARS-Zimbabwe	Introgressed lines	0.94
29	RARS_4	RARS-Zimbabwe	Introgressed lines	1.00
30	RARS_5	RARS-Zimbabwe	Introgressed lines	0.99
31	RARS_6	RARS-Zimbabwe	Introgressed lines	0.98
32	RARS_7	RARS-Zimbabwe	Introgressed lines	0.96
33	RARS_8	RARS-Zimbabwe	Introgressed lines	0.58
34	RARS_9	RARS-Zimbabwe	Introgressed lines	0.99
35	RARS_11	RARS-Zimbabwe	Introgressed lines	0.98
36	RARS_12	RARS-Zimbabwe	Introgressed lines	1.00
37	RARS_16	RARS-Zimbabwe	Introgressed lines	0.95
38	RARS_17	RARS-Zimbabwe	Introgressed lines	0.96
39	RARS_18	RARS-Zimbabwe	Introgressed lines	0.94
40	RARS_19	RARS-Zimbabwe	Introgressed lines	0.96
41	RARS_20	RARS-Zimbabwe	Introgressed lines	0.98

Introgressed - tropical maize inbred lines introgressed with genes from temperate germplasm

Table 2. 2: Description of maize inbred lines used in the study

Entry	Code	Breeding Environment	Germplasm background	Homozygosity
42	RARS_21	RARS-Zimbabwe	Introgressed lines	0.93
43	RARS_22	RARS-Zimbabwe	Introgressed lines	0.89
44	RARS_23	RARS-Zimbabwe	Introgressed lines	1.00
45	RARS_24	RARS-Zimbabwe	Introgressed lines	1.00
46	RARS_25	RARS-Zimbabwe	Introgressed lines	1.00
47	RARS_26	RARS-Zimbabwe	Introgressed lines	0.99
48	RARS_27	RARS-Zimbabwe	Introgressed lines	1.00
49	RARS_28	RARS-Zimbabwe	Introgressed lines	0.99
50	RARS_29	RARS-Zimbabwe	Introgressed lines	1.00
51	DLMF7_3	URS-South Africa	Introgressed lines	0.99
52	DLMF7_7	URS-South Africa	Introgressed lines	1.00
53	DLMF7_14	URS-South Africa	Introgressed lines	0.59
54	DLMF7_17	URS-South Africa	Introgressed lines	0.95
55	DLMF7_20	URS-South Africa	Introgressed lines	1.00
56	DLMF7_28	URS-South Africa	Introgressed lines	0.99
57	DLMF7_30	URS-South Africa	Introgressed lines	0.98
58	DLMF7_33	URS-South Africa	Introgressed lines	0.96
59	DLMF7_38	URS-South Africa	Introgressed lines	0.98
60	DLMF7_41	URS-South Africa	Introgressed lines	0.95
61	DLMF7_45	URS-South Africa	Introgressed lines	0.99
62	DLMF7_49	URS-South Africa	Introgressed lines	1.00
63	DLMF7_51	URS-South Africa	Introgressed lines	1.00
64	DLMF7_53	URS-South Africa	Introgressed lines	1.00
65	DLMF7_54	URS-South Africa	Introgressed lines	0.68
66	DLMF7_59	URS-South Africa	Introgressed lines	0.99
67	DLMF7_65	URS-South Africa	Introgressed lines	0.99
68	DLMF7_72	URS-South Africa	Introgressed lines	0.99
69	DLMF7_79	URS-South Africa	Introgressed lines	1.00
70	DLMF7_84	URS-South Africa	Introgressed lines	1.00
71	DLMF7_88	URS-South Africa	Introgressed lines	0.81
72	DLMF7_90	URS-South Africa	Introgressed lines	0.99
73	DLMF7_93	URS-South Africa	Introgressed lines	0.62
74	DLMF7_96	URS-South Africa	Introgressed lines	0.99
75	DLMF7_112	URS-South Africa	Introgressed lines	0.95
76	DLMF7_124	URS-South Africa	Introgressed lines	0.99
77	TE36	URS-South Africa	Temperate	1.00
78	TE101	URS-South Africa	Temperate	0.98
79	TE102	URS-South Africa	Temperate	0.99
80	TE115	URS-South Africa	Temperate	0.96
81	TE92	URS-South Africa	Temperate	1.00
82	TE33	URS-South Africa	Temperate	0.99

Introgressed - tropical maize inbred lines introgressed with genes from temperate germplasm; temperate, temperate germplasm background

Table 2. 3: Description of maize inbred lines used in the study

Entry	Code	Breeding Environment	Germplasm background	Homozygosity
83	DTAB_93	URS-South Africa	Temperate	1.00
84	DTAB_49	URS-South Africa	Temperate	0.99
85	DTAB_28	URS-South Africa	Temperate	1.00
86	DTAB_15	URS-South Africa	Temperate	0.99
87	DTAB_104	URS-South Africa	Temperate	1.00
88	DTAB_103	URS-South Africa	Temperate	1.00
89	DTAB_19	URS-South Africa	Temperate	0.96
90	DTAB_1	URS-South Africa	Temperate	0.98
91	DTAB_30	URS-South Africa	Temperate	1.00
92	DTAB_105	URS-South Africa	Temperate	0.99
93	DTAB_45	URS-South Africa	Temperate	0.98
94	DTAB_59	URS-South Africa	Temperate	0.94
95	DTAB_69	URS-South Africa	Temperate	0.98
96	DTAB_39	URS-South Africa	Temperate	1.00
97	DTAB_111	URS-South Africa	Temperate	0.99
98	DTAB_22	URS-South Africa	Temperate	0.94
99	DTAB_41	URS-South Africa	Temperate	0.99
100	DTAB_118	URS-South Africa	Temperate	1.00
101	DTAB_114	URS-South Africa	Temperate	0.96
102	08CED6_7_B	URS-South Africa	Temperate donor parent line	0.95
103	SC01	RARS-Zimbabwe	Tropical	1.00
104	SC02	RARS-Zimbabwe	Tropical	0.72
105	SC03	RARS-Zimbabwe	Tropical	1.00
106	SC04	RARS-Zimbabwe	Tropical	0.98
107	SC05	RARS-Zimbabwe	Tropical	1.00
108	SC06	RARS-Zimbabwe	Tropical	1.00
109	SC07	URS-South Africa	Tropical	1.00
110	SC08	URS-South Africa	Tropical	1.00
111	SC09	RARS-Zimbabwe	Tropical	0.95
112	SC10	RARS-Zimbabwe	Tropical	1.00
113	SC11	RARS-Zimbabwe	Tropical	0.99
114	SC12	RARS-Zimbabwe	Tropical	1.00
115	SC13	RARS-Zimbabwe	Tropical	0.99
116	SC14	RARS-Zimbabwe	Tropical	0.64
117	SC15	RARS-Zimbabwe	Tropical	1.00
118	SC16	RARS-Zimbabwe	Tropical	0.99
119	SC17	RARS-Zimbabwe	Tropical	1.00
120	SC18	RARS-Zimbabwe	Tropical	1.00
121	SC19	RARS-Zimbabwe	Tropical check inbred line	1.00
122	SC20	RARS-Zimbabwe	Tropical check inbred line	0.96
123	SC21	RARS-Zimbabwe	Tropical check inbred line	0.95

Introgressed -tropical maize inbred lines introgressed with genes from temperate germplasm, temperate, temperate germplasm background, and tropical-tropical germplasm lines recipient parents. Homozygosity of the lines obtained using SNP markers

2.2   Environments

The research environments used for the study are described in Table 2.4.

2.3   Experimental design

Experiments were designed as augmented alpha lattice designs (Lin and Poushinsky, 1983; Scott and Miliken, 1993; Spehar, 1994) with common checks and replication over sites in both trials. The large number of experimental entries involved in the trials required the use of an augmented design as large numbers of entries made it difficult to conduct trials because of challenges of: environmental heterogeneity in the field that could not easily be taken into account, and limitation of seed availability for each experimental entry. Hence the design allowed the check entries which had enough seed available to be repeated several times in each block. Each repetition of the check entry was randomly assigned to plots embedded in a block and experimental entries were also randomly assigned to plots that were not allocated to check entries to give unbiased error estimates. Entries (experimental and check entries) were randomly assigned into blocks. Estimation of block effects and plot error was done only with respect to check entries. The estimated block effects were used to adjust the observed values of the experimental entries. The error was used to test for significance of experimental entry differences. Detailed description of each experimental design is presented in each respective chapter in the thesis.

Table 2. 4: Description of test and selection environments used in the study

Location	Country	Latitude	Longitude	Altitude(m)	Description (units)	A	B	Type of stress
					Season data			
*RARS	Zimbabwe	17°14'E	31° 14'E	1300	Av max temp (°C)	26.7	28.6	Gray leaf spot (GLS)
					Av min temp (°C)	12.5	12.8	Northern leaf corn blight (NCLB)
					Rainfall (mm)	865.0	918.0	Common Rust (Rust)
								Phaeosphaeria leaf spot (PLS)
								Ear rots
								Maize streak virus (MSV)
								Short day length
*KRC	Zimbabwe	18°16'S	29°50'E	1149	Av max temp (°C)	22.1	28.1	Heat and drought stress
					Av min temp (°C)	15.5	20.4	Short day length
					Rainfall (mm)	416.4	724.0	
**Ukulinga	South Africa	29°37'S	30°16'E	812	Av max temp (^{o}C)	25.9	24.0	Heat and drought stress
					Av min temp (^{o}C)	16.0	12.9	Increased cold soil temperature
					Rainfall (mm)	600.7	885.0	Increased frost exposure
								Gray leaf spot (GLS)
								long day length
**Cedara	South Africa	26°32'S	30°16'E	1068	Av max temp (°C)	25.2	23.6	Northern leaf corn blight (NCLB)
					Av min temp (°C)	13.0	9.6	Phaeosphaeria leaf spot (PLS)
					Rainfall (mm)	647.0	873.0	Gray leaf spot (GLS)
								long day length
**Potchefstroom	South Africa	26°73'S	27°75'E	1349	Av max temp (^{o}C)	27.7	25.7	Heat and drought stress, rain poor distributed in the season
					Av min temp (°C)	19.5	9.8	Phaeosphaeria leaf spot (PLS)
					Rainfall (mm)	708.7	703.1	long day length

RARS-Rattray Anorld Research Station; KRC-Kadoma Research Centre; Ukulinga-Ukulinga Research Station; Cedara-Cedara Research Station; Potchefstroom-Potchefstroom Research Station; m.a.s.l, meters above sea level; **A**-2012-13 season data; **B**-Long term average seasonal data; Av-Average;**Weather data provided by the Agricultural Research Council – Institute for Climate, Soil and Water; * Weather data provided by CIMMYT's network of automatic weather stations.

2.4 Field layout and management

In South Africa, at Ukulinga Research Station each entry was planted to single row plots of 5m length, spaced at 0.3m in-row and 0.75m between row spacing to achieve a total plant population density of at least 44 000 plants ha^{-1}. At Cedara Research Station, single 5m row plots, in-row spacing 0.3 and inter-row 0.9m were used to achieve a plant stand of at least 37 000 plants ha^{-1}. At Potchefstroom Research Station, single row plots of 6.6m length, spaced at 0.25m in-row and 1.5m between row spacing to attain a total plant population density of at least 26 000 plants ha^{-1}. In Zimbabwe, at Rattray Anorld Research Station and Kadoma Research Centre each entry was planted to single row plots of 10m length, space at 0.3m in-row and 1.5m between row spacing to achieve a total plant population density of at least 22 000 plants ha^{-1}. Inter-row differences were due to agronomic practice implemented at each site. Standard cultural management practices for growing maize were carried out at all sites. Irrigation was only applied to achieve uniform establishment and also to supplement rainfall as and when necessary. Fertilizer application was done at a rate of: 120kg Nitrogen (N), 33kg Phosphorous (P), and 44kg Potassium (K) at Cedara, Ukulinga and Potchefstroom Research Stations; 145kg Nitrogen (N), 56kg Phosphorous (P), and 28kg Potassium (K) at Rattray Anorld Research Station; and 88.4 kg Nitrogen (N), 56kg Phosphorous (P), and 28kg Potassium (K) at Kadoma Research Centre. Fertilizer recommendations were based on long term agronomic practices at each site.

2.5 Variables measured

Comprehensive data was collected following standard procedures used at CIMMYT (1985) for the following traits:

a) Anthesis days (AD): number of days to 50 % pollen shedding from day of planting.
b) Silking days (SD): number of days to 50% silk emergence from day of planting.
c) Plant height (m) (PH): distance between the base of a plant to the auricle of the flag leaf.

d) Ear height (m) (EH): distance between the ground level and the base of the primary ear.

e) Stalk lodging (SL): percentage of plant per plot that had their stems broken.

f) Root lodging (RL): percentage of plant per plot which had their stems inclined by at least 45°.

g) Number of ears per plant-Ear prolificacy (EPP): count of number of ears plot as a fraction of number of plants.

h) Moisture content at harvest (MC): percentage grain moisture content at harvest.

i) Grain yield (t ha^{-1}) (GYD): grain mass per plot adjusted to 12.5 % moisture content**.**

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3 Molecular characterisation of maize introgressed lines bred in different environments

Abstract

Establishing detailed information of genetic diversity and relationship of germplasm in a breeding programme ensures effective utilization of germplasm. Introgression of temperate germplasm into tropical elite inbred lines using a common donor inbred line and different selection environments might disrupt the heterotic grouping system. Therefore, the objective of the study was to determine the effect of introgression and selection environments on the clustering pattern of the new introgressed lines. A total of 123 maize inbred lines that were derived by introgression of temperate germplasm into tropical elite inbred lines, and four generations of pedigree selection in three distinct environments in Zimbabwe and South Africa were characterised using 20 SSR markers. It was observed that the 20 SSR markers were effective in discriminating the introgressed lines according to genetic distance and clustering. A total of 83 alleles were detected with an average of 4.15 alleles per locus, allelic diversity of 0.53 and PIC of 0.47. Introgression of temperate germplasm into tropical elite inbred lines did not disrupt heterotic groupings because introgressed lines were inclined towards the original heterotic groups from which they were derived. However, there were some introgressed lines (14%) that did not show any such orientation. Future similar studies should use a higher number of SSR markers. There was genetic diversity among introgressed lines developed in the same environment, and they were all clearly different from the founder parents. Furthermore, selection environments did not influence clustering of introgressed lines.

Keywords: maize breeding, introgression, selection environment, simple sequence repeats (SSR) markers.

3.1 Introduction

Continuous development of hybrid maize varieties that are highly adaptable and productive in target environments requires maintenance of large genetic diversity, and distinct heterotic clusters. In maize breeding programmes, knowledge of genetic diversity and relationship among breeding materials is crucial for: planning crosses in hybrid production; line development and heterotic grouping; germplasm conservation and management (Patto et al., 2004). This ensures an efficient breeding programme that effectively uses resources in maximizing heterosis in hybrid combinations. Heterosis or hybrid vigour is the better performance of a hybrid relative to the parents, and is the outcome of genetic and phenotypic variation (Ali et al., 2012). Manifestation of heterosis in any breeding programme is dependent on the genetic divergence of parental lines used (Hallauer and Miranda, 1988). Hence there is need to have knowledge of genetic diversity of breeding material if maximum hybrid vigour is to be realised.

An effective maize hybrid programme requires the need to establish and maintain genetic divergence between germplasm groups thus ensuring significant improvement in agronomic and yield potential. In the current study temperate germplasm was introgressed into tropical elite inbred lines in a manner that might have compromised genetic diversity. A single common donor parental inbred line was used to introgress temperate germplasm in 12 elite tropical inbred lines from different tropical heterotic groups. However the resulting breeding populations were allowed to go through a recombination process at F_2 and F_3 and maximum transgressive segregation (selection for genetic outliers) before pedigree selection was applied at three different environments. Breeding programmes can control recombination in population development; however the level of effect of this control on genetic variation and selection response is still debatable. A number of studies have proffered that maximizing recombination during population development will increase genetic variation and selection gains (Wijnker and de Jong, 2008; McClosky and Tanksley, 2013).

In this study, a single seed descent from genetic outliers (transgressive segregants) was utilized to develop introgressed lines. Therefore there was expected difference in genetic diversity for the introgressed lines despite the use of a common donor parental inbred line. It was thus prudent to establish the effect of introgression on the established heterotic system and genetic diversity, and the resultant effect of selection environments on both genetic diversity and clustering pattern.

Tropical and temperate maize germplasm can be exploited to enhance maize hybrids in breeding programmes. According to Prasanna (2012), genetic diversity in maize across temperate and tropical germplasm offers possible opportunities for genetic enhancement and increase in maize yield production across environments (Prasanna, 2012). However, several studies (Tallury and Goodman, 1999; Abadassi and Herve, 2000; Tarter et al., 2004; Reif et al., 2010) have reported lack of adaptability of tropical germplasm when directly introduced in temperate environments. Breeding programmes operating from tropical environments have also directly introduced their tropical elite maize germplasm into the South African warm temperate environments. This has been characterized by lack of adaptability. Undesirable traits such as late flowering, excessive rank growth and lodging, high grain moisture content at harvest, poor plant standing ability, and low grain yield potential (Tarter et al., 2004) have been exposed under temperate environments. Therefore it was sensible in the current study to introgress temperate germplasm into tropical elite inbred lines to improve these traits.

Introgression of genes from exotic maize germplasm has been explored as an effective breeding strategy for enhancing genetic diversity and sustained increased productivity. A number of studies have reported introgression of exotic maize germplasm for improving adaptability, yield potential and genetic diversity in temperate germplasm as a promising approach for improving desired economic traits (Goodman, 1999; Tarter et al., 2004; Nelson et al., 2006; Nelson and Goodman, 2008; Reif et al., 2010; Wang et al., 2008). However, there is poor documentation of the information regarding the effective use of temperate maize germplasm as a source of desirable genes in tropical germplasm (Goodman, 1999; Abadassi and

Herve, 2000). Therefore there is the need to explore the potential of temperate germplasm as a source of exotic genes in tropical germplasm to enhance adaptability to the South African warm temperate environments. Genetic diversity generated in introgressed lines may be utilized to create new hybrids that are adaptable and also with high heterosis in temperate environments. However, this suggestion is based on the assumption of a positive association between genetic distance and heterosis.

A number of techniques have been utilized to characterize genetic diversity in maize germplasm. Patto et al. (2004), reports that genetic diversity can be characterized using pedigree; phenotypic and molecular data. Unfortunately, morphological data may be greatly influenced by environmental effects and pedigree information may not be available, incomplete, or unreliable for all the materials in the programme. Therefore, there is need to use molecular markers that are more reliable and precise to characterize maize inbred lines at the DNA sequence level. Molecular markers are not influenced by environmental factors and provide a powerful tool that also allows characterization of a greater number of inbred lines, thus potentially increasing efficiency of maize breeding programmes (Ahmad et al., 2011). This only applies well where there is positive correlation between molecular and phenotypic diversity. According to Xiao et al. (1996) and Zhang et al. (1996), there is variable relationship between molecular and phenotypic diversity depending on genetic material used, diversity of germplasm and complexity of genetic basis of heterosis.

Establishment of genetic potential of economic traits of interest in maize breeding during selection requires optimum conditions, thus ensuring selection of inbred lines that are unique to a defined selection environment. Indirect selection is considered effective for selecting traits that have high heritability across environments. However, this is not always the case for complex traits due to genotype-by-environment interactions. Hence there is the need to select genotypes in their target environment through the use of different selection environments. In the current study, three selection environments were used to ensure expression of the genetic potential of the introgressed lines in each selection environment. Therefore, in the current study it

was necessary to establish the effect of the selection environment on genetic diversity and heterotic clustering of the introgressed lines.

The major objectives of this study were two-fold. Firstly, to determine effects of introgressing temperate germplasm into tropical elite inbred lines on genetic diversity and clustering patterns of introgressed lines relative to the founder parents and the public heterotic systems. Secondly, to determine the effects of selection environments on genetic diversity and clustering pattern of introgressed lines.

3.2 Materials and Methods

3.2.1 Germplasm

A total of 123 maize inbred lines consisting of 76 introgressed lines, 26 temperate parental inbred lines and 21 elite tropical parental inbred lines. The germplasm was developed as described in Chapter 2.

3.3 Genotyping

3.3.1 Tissue sampling

At four weeks after planting, leaf sample tissue of each genotype was harvested from the inbred yield trial (see Chapter 4) that was planted at Rattray Anorld Research Station in Zimbabwe using the Punch method (http//:www.dnalandmarks.ca Accessed 18 September 2013). The leaf discs (average of 10 discs) of each genotype were placed into two labelled 96-well blocks with each well representing each individual genotype. Blocks were sealed using an air-pore tape and placed inside plastic bags together with 50g of silica gel to dry them. Samples were then shipped to the DNA Landmarks laboratory in Canada. The sampling method used was recommended by the service provider (http//:www.dnalandmarks.ca Accessedn18 September 2013).

3.3.2   DNA extraction and isolation

Deoxyribonucleic acid (DNA) was extracted following a proprietary Sarkosyl Nitrogen based method at the DNA Landmarks laboratory (http//:www.dnalandmarks.ca Accessed 18 September 2013).

3.3.3   Genotypic data analysis

A total of 20 SSR markers based on the previous research studies on maize at University of KwaZulu-Natal (see Appendix 3.1 and Appendix 3.2) were used for polymerase chain reaction (PCR) amplification. These SSR markers were chosen on the basis of bin location (to maximize genomic coverage) (www.dnalandmarks.c.a Accessed on 18 September 2013) such that there were two markers per chromosome. Polymerase chain reactions (PCR) were performed using DNA landmark standard procedure (www.dnalandmarks.ca Accessed on 18 September 2013). The SSR gel images and marker data were processed using Gene Mapper V40 Software (www.genemapperV40.com Accessed on 18 September 2013). Failed samples were repeated at least once. The software was used to score genetic distances and allele sizes in which bands were measured then dually coded by 1 or 0 for their presence or absence, respectively, and -1 for missing data. PowerMaker V3. 25 (Liu, 2004) molecular analysis software package was used for calculating; similarity matrix based on Nei method (Nei, 1983) using coded data, and cluster analysis based on similarity matrices obtained with Unweighted Pair Group Method with Arithmetic (UPGMA) to generate dendrograms.

Data was analysed for the whole sample of 123 maize inbred lines to determine clustering pattern of new introgressed lines relative to the standard inbred lines and founder parents. Then the data was analysed in three subsets of the introgressed lines. The data subsets were a) 20 SSR markers x 25 introgressed lines bred at Kadoma Research Centre in Zimbabwe; b) 20 SSR markers x 25 introgressed lines bred at Rattray Anorld Research Station in Zimbabwe, c) 20 SSR markers x 26

introgressed lines bred at Ukulinga Research Station in South Africa. The data for the common parent was included in each subset.

The following parameters were calculated to characterize the SSR markers:

a) Polymorphic information content (PIC) formula (Botstein et al., 1980) was used to calculate values at each locus as:

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

PIC values give an estimate of the discriminatory power of a marker by taking into account not only the number of alleles at the locus but also the relative frequencies of these alleles, *where:* $\acute{P}_u{}^2$ = *frequency of the marker allele, k = number of alleles,* $P^2{}_u$ = *frequency of the uth marker, $P^2{}_v$= frequency of vth allele.*

b) Allelic diversity formula (Singh, 2006) was used to calculate values as:

$$\text{Div} = 1 - \sum_{u=1}^{k} \tilde{p}_u^2$$

Where k = number of alleles, $\acute{P}u^2$ = frequency of the marker allele.

3.4   Results

3.4.1 Marker Characterization

The 20 SSR markers were found suitable and very effective for characterization of the lines. All twenty SSR marker loci were polymorphic across the 123 maize inbred lines and a total of 83 alleles were detected. Table 3.1 shows the number of alleles scored across the SSR loci,   which ranged from 2 to 6, and an average number of 4.15 alleles per locus. Allelic diversity indices of each locus ranged from 0.09 (nc133) to 0.75 (PHI102228) with a mean of 0.53.   Maximum number of alleles that was detected by the markers was six and this was detected at four loci namely PHI102228, PHI308707, UMC1161, UMC1545. Minimum number of alleles detected

was two at the PHI062 locus. The shortest marker size range was from UMC1545 (66-79) with the longest being PHI056 (237-255). The PIC estimates ranged from 0.09 to 0.71 with a mean of 0.47. Repeat units ranged from 2-8, with tri and tetra nucleotide motifs as the most abundant, seven and eight, respectively. The tri nucleotide motifs represented 35% and tetra 40%; while the remaining 25% was represented by di, penta and hexa nucleotide motifs. Heterozygosity values ranged between 0.11 (PHI123) and 0.90 (nc130) with a low mean value of 0.20. Seven SSR loci: PHI056, PHI072, PHI102228, PHI114, PHI308707, UMC1367 and UMC1545 had PIC value of equal or more than 0.6, showing their potential to detect differences among inbred lines. Profiles of the twenty markers were collectively able to discriminate all the inbred lines (Figure 3.1).

3.4.2 Effect of introgression and selection environment on genetic clusters

A radial dendrogram, a visualization which enables presentation of a large number of germplasm was used to present genetic diversity data (Figure 3.1). The main divergence on the dendrogram occurred at genetic distances 0.38 with the 123 maize inbred lines being divided into two major clusters (1 and 2). Environment from which introgressed lines were bred from did not influence clustering of the 76 introgressed lines. There was a random allocation of introgressed lines to different genetic clusters.

A total 106 (86%) of introgressed lines were in alignment with the heterotic groups of their founder parents; whereas 14% of the new introgressed lines were non-aligned. Heterotic group classification of inbred lines in eastern and southern Africa was represented by tropical lines related to Southern Cross (SC), Salisbury White (N3), K64r, B17 and Natal Potchefstroom Pearl Elite Selection (NPPES/P) populations. In this study, heterotic orientation of the introgressed lines could be established based on these public heterotic groupings. Some of the introgressed lines were placed in the same cluster with lines of the following heterotic orientation; N3, SC, K64R, B17 and P (NPPES) as described in the following sections (Figure 3.1).

Table 3. 1: Characterization of 123 maize inbred lines using 20 SSR markers

SSR locus	Repeat unit	Chromosome	No. of Alleles	Frequencies	Allelic Diversity	Heterozygosity	Size Range	PIC
PHI029	AGCG -tetra	3	4	0.32	0.48	0.13	148-160	0.4
PHI031	GTAC-tetra	6	4	0.69	0.47	0.13	192-229	0.43
PHI056	CCG-tri	1	5	0.01	0.68	0.16	237-255	0.62
PHI062	ACG-tri	10	2	0.55	0.49	0.07	158-161	0.37
PHI065	CACTT-penta	3	4	0.52	0.53	0.18	136-157	0.42
PHI072	AAAC-tetra	4	5	0.25	0.65	0.12	147-168	0.6
PHI075	CT-di	6	4	0.05	0.55	0.16	225-239	0.46
PHI084	GAA-tri	10	3	0.07	0.31	0.03	155-161	0.28
PHI102228	AAGC-tetra	3	6	0	0.75	0.73	110-132	0.71
PHI112	AG-di	2	3	0.13	0.25	0.04	135-157	0.22
PHI114_tailed	GCCT-tetra	7	5	0.39	0.7	0.15	135-167	0.65
PHI123	AAAG-tetra	4	3	0.33	0.5	0.11	151-156	0.41
PHI308707	AGC-tri	6	6	0.01	0.72	0.13	109-128	0.67
PHI331888	AAG-tri	5	4	0.09	0.56	0.12	130-136	0.5
UMC1161	GCTGGG-hexa	8	6	0.04	0.45	0.09	132-150	0.42
UMC1304	TCGA-tetra	8	3	0.01	0.51	0.15	116-134	0.38
UMC1367	CGA-tri	10	4	0.32	0.66	0.30	140-159	0.6
UMC1545	AAGA-tetra	4	6	0.24	0.72	0.16	66-79	0.68
nc130	AGC-tri	5	3	0.51	0.51	0.90	147-153	0.39
nc133	GTGTC-penta	2	3	0.01	0.09	0.04	107-113	0.09
Mean			4.15	0.23	0.53	0.20		0.47

3.4.2.1   Salisbury White (N3) heterotic group

Four (6%) introgressed lines were inclined towards known tropical inbred lines: SC09, SC07 and SC08 (sub cluster 2A); SC4 (sub cluster 2I); and SC10, SC11, SC14, SC16, SC20 and SC21 (sub cluster 2k) which belong to the heterotic grouping N3. Sub cluster 2A also had introgressed line RAFS_16 that was bred at Rattray Anorld Research Station in Zimbabwe. Sub cluster 2I had introgressed lines bred from all the selection environments. This sub cluster also included the donor parental inbred line. The sub cluster 2K contained introgressed lines bred from two of the three selection environments except Kadoma Research Centre in Zimbabwe (Figure 3.1).

3.4.2.2   Southern Cross (SC) heterotic group

The highest number 42 (55%) of introgressed lines were inclined towards the SC heterotic grouping with reference to known tropical inbred lines: SC02 (sub cluster 2B); SC13 (sub cluster 2D); SC03 (sub cluster 2F) and SC05 (sub cluster 2H). The composition of sub clusters 2B was nine introgressed lines bred from all three selection environments. In sub cluster 2D, entry KRC_1 bred from Kadoma Research Centre in Zimbabwe was the only introgressed line present. Seven introgressed lines bred from all selection environments were placed in sub cluster 2F. The highest number of introgressed lines (25) was placed in cluster 2 (Figure 3.1).

3.4.2.3   Natal Potchefstroom Pearl Elite Selection (P) heterotic group

Sub cluster 2C had tropical parental inbred lines SC18, SC15 and SC19 all from the P heterotic grouping and eight (11%) introgressed lines namely; KRC_5, KRC_23, KRC_38, KRC_41, KRC_43 bred from Kadoma Research Centre in Zimbabwe, RAFS_1 bred from Rattray Anorld Research Station in Zimbabwe), and DLMF7_14, DLMF7_17 and DMLF_112 bred from Ukulinga Research Station in South Africa (Figure 3.1).

3.4.2.4   K64r heterotic group

A known tropical inbred line, SC12 (sub cluster 2E), which is a K64r derivative and therefore represented the K64r heterotic grouping was placed in this cluster. This cluster was dominated by 21 temperate inbred lines that were inclined towards K64r heterotic group. However, there were no (0%) introgressed lines that were placed in this cluster and heterotic grouping (Figure 3.1).

3.4.2.5   B17 heterotic group

B17 heterotic group derived from tropical germplasm lines related to CML202 population which is classified by CIMMYT as heterotic group A, had 10 (14 %) introgressed lines in sub cluster 2J namely: KRC_24 and KRC_27 bred from Kadoma Research Centre in Zimbabwe; RARS_4, RARS_11, RARS_12, RARS_17, RARS_24 bred from Rattray Anorld Research Station in Zimbabwe); and DMLF_53, DMLF_59 and DMLF_88 (Ukulinga Research Station in South Africa) were inclined towards this heterotic grouping. This cluster also included one temperate inbred line DTAB_93 (Figure 3.1).

3.4.2.6   Non-aligned introgressed lines

Eleven (14%) of the introgressed lines from three clusters did not show any orientation towards the public heterotic groups. Cluster 1 had maize inbred lines bred from all three selection environments namely: 1A Rattray Anorld Research Station in Zimbabwe (RAFS_8) and; 1B Ukulinga Research Station in South Africa (DLM7_93) and Kadoma Research Centre in Zimbabwe (KRC 7). None of the founder parents were placed in this cluster. Sub cluster 2G had RAFS_21 and RAFS_22 bred from Rattray Anorld Research Station in Zimbabwe, KRC_4 bred from Kadoma Research Centre in Zimbabwe, and DMLF7_20 bred from Ukulinga Research Station in South Africa. In sub cluster 2L there was RAFS_3 bred from Rattray Anorld Research

Station in Zimbabwe, KRC_8 bred from Kadoma Research Centre in Zimbabwe and a temperate inbred line DTAB_22 (Figure 3.1).

Figure 3. 1: Radial dendrogram of 123 maize inbred lines selected at 3 environments (Rattray Anorld Research Station-Zimbabwe, Kadoma Research Centre-Zimbabwe and Ukulinga Research Station-South Africa).

3.4.3 Genetic diversity among introgressed lines developed in the same environment

The cluster analysis indicated that there was genetic variation among introgressed lines that were developed at the same site, and that all the lines were different from the donor parent. The data is presented in Figures 3.2 through 3.4. The 26 germplasm lines which were bred at Ukulinga Research Station in South Africa (Figure 3.2) were grouped into two major clusters 1 and 2. Cluster 1 had one inbred line DLMF7_93. Cluster 2 had five sub-clusters which indicated distinct groupings within this cluster. Donor parental inbred line (08CED6_7_B) was placed in sub cluster 2D which had the highest number of inbred lines (9).
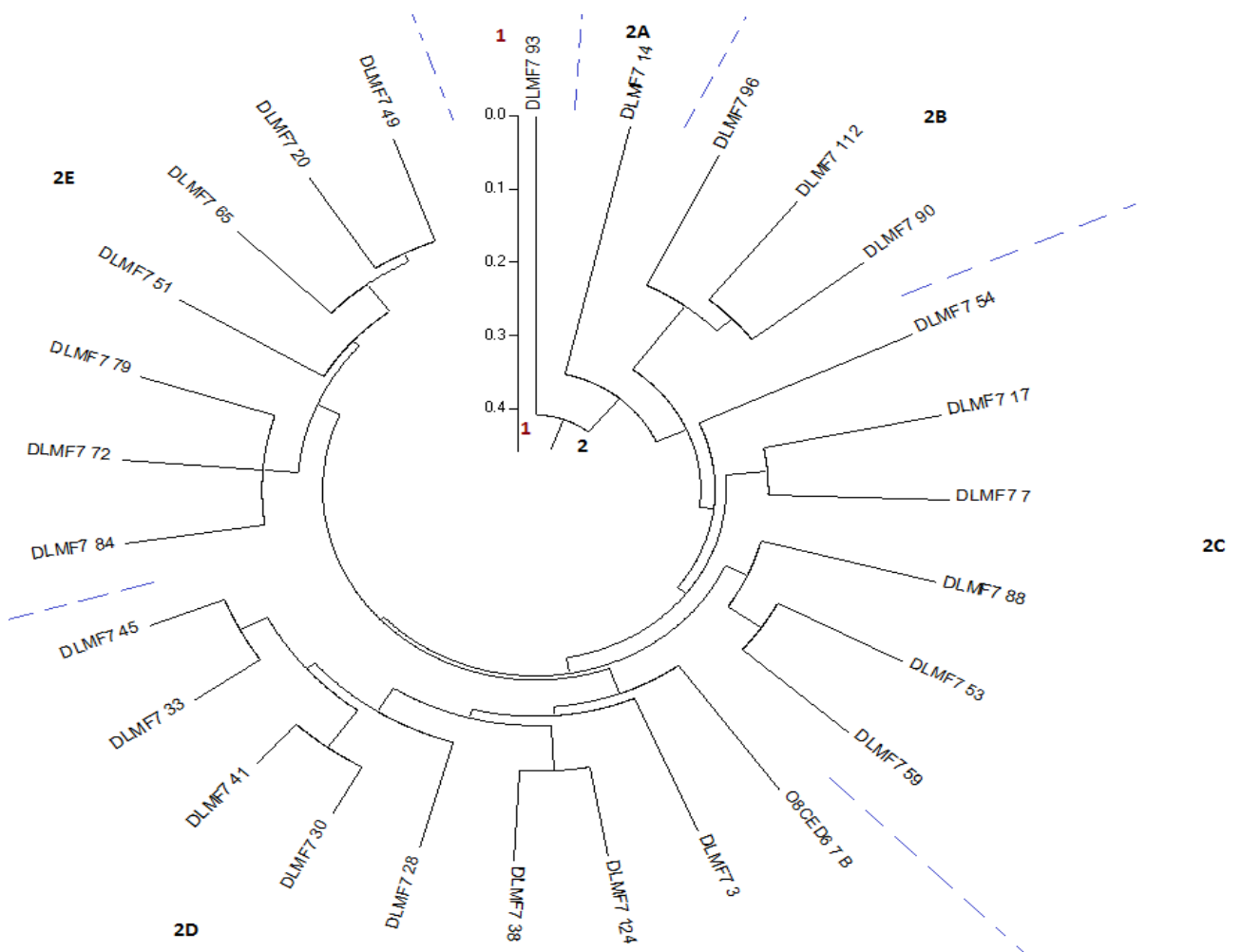
Figure 3. 2: Radial dendrogram of 26 introgressed lines bred at Ukulinga Research Station

Figure 3.3 shows a radial dendrogram of cluster analysis of introgressed lines sets bred from Kadoma Research Centre in Zimbabwe. The 25 inbred lines were grouped into two major clusters (1 and 2). Cluster 1 had two sub clusters 1A (KRC_7) and 1B (KRC_43, 23 and 41). In cluster 2, there were seven sub-clusters, with the donor parental line (08CED6_7_B) placed in sub cluster 2F. Sub cluster 2E had the highest number of inbred lines (8).
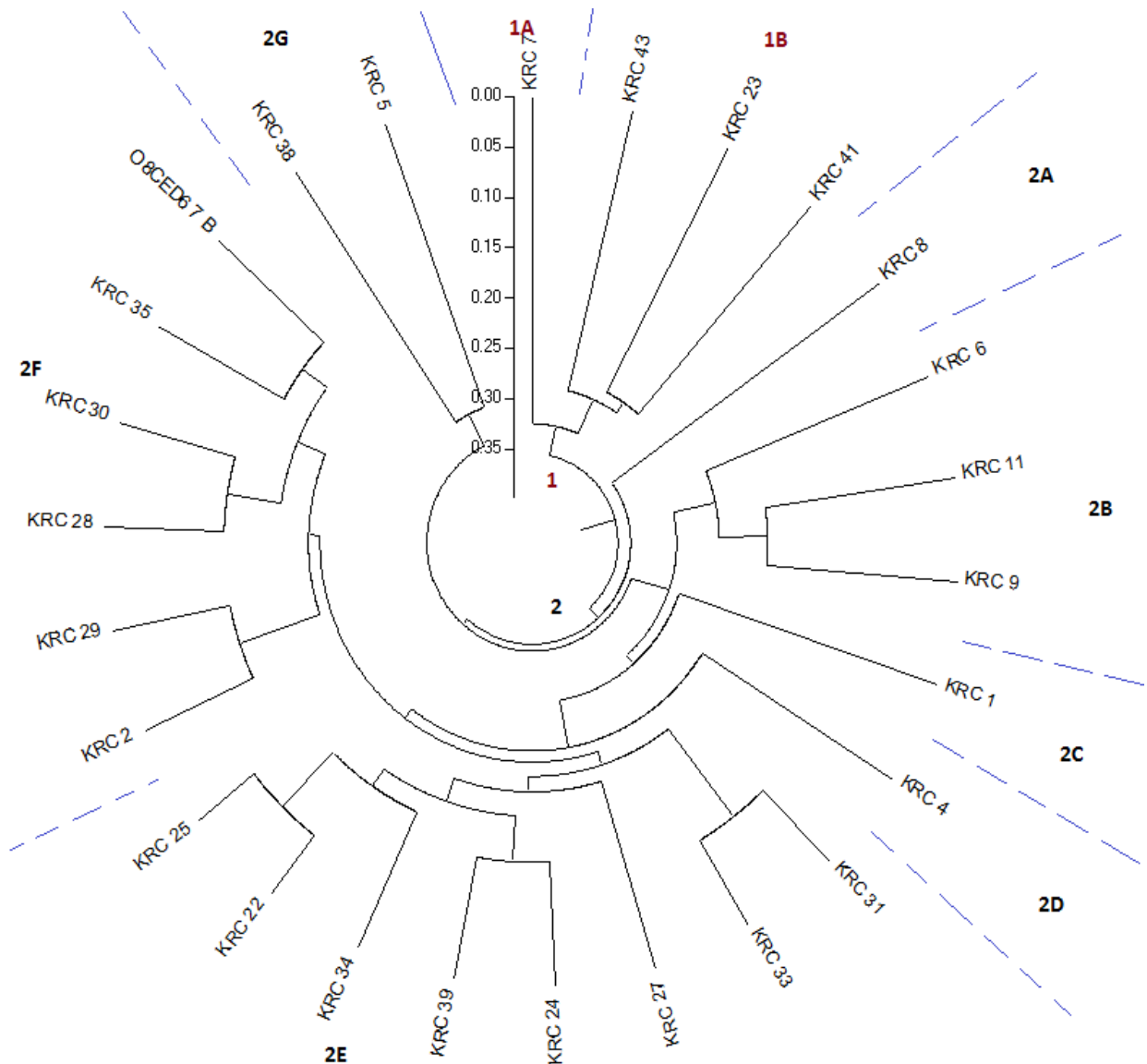
Figure 3. 3: Radial dendrogram of 25 introgressed lines bred at Kadoma Research Centre

There were two major clusters for introgressed lines set that were bred at Rattray Anorld Research Station in Zimbabwe (Figure 3.4). Cluster 1 had two sub-clusters 1A (RAFS_1) and 1B (RAFS_8), while cluster 2 had 11 sub clusters. Donor parental inbred line (08CED6_7_B) was placed in sub cluster 2K. Sub clusters 2K and 2G had the highest number of introgressed lines (5).
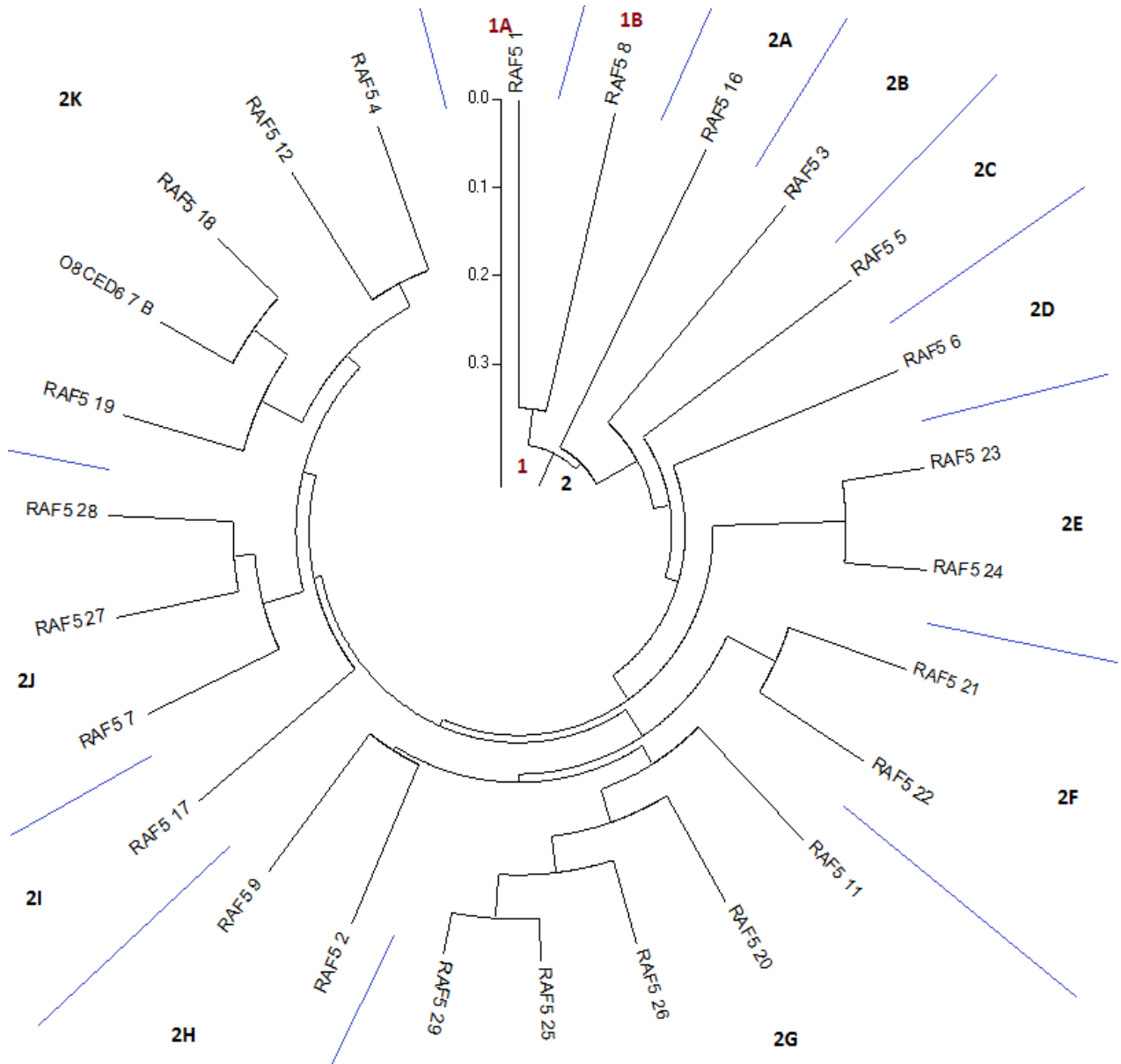
Figure 3. 4: Radial dendrogram of 25 introgressed lines bred at Rattray Arnold Research Station.

3.5   Discussion

3.5.1 Marker characterization

The average number of detected alleles per locus in this study was 4.15 alleles per locus which is comparable to those previously reported in the literature. Choukan et al. (2006) using 56 inbred lines with 46 SSR markers reported an average of 4.9 alleles per locus. However, a number of studies have reported higher average alleles per marker in maize inbred diversity studies. Zhi-zhai et al. (2010) using 143 maize landraces with 54 SSR markers reported an average of 9.57 alleles per locus with a range of 4 to 22. Even though the sample size was large in this study, there was a lower average number of alleles per locus and allelic diversity indices of each locus ranged from 0.09 to 0.75 with a mean of 0.53. This can partly be explained by involvement of introgressed lines with a common temperate parent inbred line (08CED6_7_B) which causes close relatedness of introgressed lines. In addition, tropical recipient lines were from one breeding programme and breeders are known to recycle germplasm during breeding which can also account for the close relatedness.

Four SSR primers namely, PHI102228, PHI308707, UMC1161, UMC1545 had the highest polymorphism followed by PHI056, PHI072, PHI114 for introgressed lines qualifying them as suitable markers for genetic fingerprinting. In this study, the PIC values for the 20 SSR loci ranged from 0.09 to 0.71, with a mean of 0.47. This indicates that the 20 SSR markers were able to discriminate and cover the genome uniformly for the maize inbred lines under study. According to Pabendon et al. (2004), the PIC values are dependent on the genetic diversity of the accessions chosen. Increase in polymorphism was noted in the current study due to increase in tri SSR repeat motifs. Repeat types have been reported to influence allelic differences that can be identified per microsatellite locus during screening (Wende et al., 2012), an increase in tri and tetra SSR repeat motifs tends to increase polymorphism (Sharopova et al., 2002). Low mean heterozygosity value of 0.20 and a range of 0.11

(PHI123) to 0.90 (nc130) obtained in this study was comparable to 0.37 and a range of 0.23 (nc133) to 0.65 (Φ 308707) reported by Yao et al. (2007).

3.5.2 Clustering and heterotic orientation

Introgression of temperate germplasm into tropical elite inbred lines did not disrupt heterotic grouping as indicated by introgressed lines that could be fitted into five existing public heterotic groups. The majority of the introgressed lines were inclined towards the SC heterotic grouping but none were inclined towards the K64r heterotic grouping which can be attributed to absence of tropical elite inbred lines in the recipient lines that were used for introgression. This indicates that K64r can be used as a potential tester line for the introgressed lines. However, 14% of introgressed lines did not show any inclination towards the public heterotic groups. This can be attributed to recombination that occurred during F_2-F_3 generation and transgressive segregation resulting in generation of new lines that were different from their founder tropical parents. Genetic recombination allows the production of new combinations of alleles that are related to the parental lines but differ in their genetic composition. Similar results of higher recombination from the same populations generated by single seed descent have been reported by Bordes et al. (2007). With the aid of heterotic grouping based on specific combining ability data; the new cluster of non-aligned group of introgressed lines can be exploited in maximizing heterosis during hybrid development.

The main divergence on the dendrogram occurred at genetic distance above 0.38 which is comparable to those previously reported in the literature. Choukan et al. (2006) on characterization of Asian maize inbred lines, George et al. (2004) on using SSR data to determine relationships and potential heterotic grouping for medium to late maturing Iranian maize inbred lines. However, Laborda et al. (2005) in a genetic diversity study of tropical maize germplasm failed to easily identify heterotic groups of tropical maize inbreds in a dendrogram using SSR markers. Effective and reliable discrimination of inbred lines not only helps in identification of genotypes, but also in promoting efficient utilization of genetic materials in breeding programmes (Prasanna

et al., 2004). However, it should be noted that these probable heterotic groupings identified in the current study through molecular analysis should be substantiated with heterotic groupings that are based on field performance data. Xiao et al. (1996) and Zhang et al. (1996), report that the relationship between groupings of genetic material based on molecular analysis and phenotypic field performance is variable, depending on genetic material used, diversity of germplasm and complexity of genetic basis of heterosis hence the need for field evaluation to obtain combining ability data for validation of the heterotic groupings.

3.5.3 Effect of selection environment on genetic grouping

Clustering patterns of the introgressed lines were not influenced by the selection environment. The two major clusters 1 and 2 contained introgressed lines from all the selection environments. This demonstrated that grouping was not based on the environment from which the introgressed lines were selected. When separate analyses were performed for the three selection environments used in this study, the three subsets showed that there was genetic diversity in each selection environment. This indicates that each selection environment was capable of differentiating the introgression maize inbred lines thus allowing selection to be carried out. All the environments had two major clusters, with the sub-clusters ranging from 4-11. The introgressed lines subset from Rattray Anorld Research Station in Zimbabwe displayed the highest genetic diversity which is evidenced by the highest number of sub-clusters observed. This indicates that this environment can fully discriminate maize germplasm and enhance genetic gain during selection.

3.5   Implications for Breeding

In maize breeding programmes establishment and maintainance of heterotic patterns or grouping of germplasm will ensure exploitation of maximum heterosis during development of hybrid combinations. Wende et al. (2012) reports that heterotic patterns or grouping of inbred lines generated will enable breeders to maintain and predict performance of maize hybrids to be developed from different inter crosses.

Therefore, there is increasing focus on establishing genetic diversity of maize germplasm in current breeding programmes. Accurate assessment of the levels of and patterns of genetic diversity is particularly useful in maize breeding. This ensures: selection of appropriate parental inbred lines for hybrid combinations; maintenance and broadening of the genetic base of the elite germplasm; and generation of segregating progenies with maximum genetic variability for further selection (Prasanna et al., 2004). In the current study, maximum heterosis in hybrids can be obtained from crossing introgressed lines that are in different heterotic groups. However these groupings have to be confirmed based on field performance of lines in hybrid combination. The reason being heterosis can also be found for inbred lines in the same heterotic group or cluster.

Probable heterotic patterns noted based on the SSR markers, must be further evaluated in the field to establish specific combing ability (SCA) that can be used to accurately classify the inbreds into heterotic groupings. This will ensure an efficient breeding programme resulting in reduced time and cost of hybrid evaluation trials. Crosses within the clusters can be used for population improvement in breeding programmes and also segregating progenies within the introgressed lines can be used for further studies on adaptation of this germplasm in South African warm temperate environments. Breeder's experience has showed that new lines can be derived from crosses between inter groups within a heterotic group (Zhang et al., 2004). Therefore in this study maximum heterosis or hybrid vigour can be obtained from crosses that have the lowest similarity coefficient value, while further line improvement can be obtained from crossing lines within the same cluster with highest similarity coefficient value. This will only fully apply if the inbred lines are to be further evaluated through quantitative cluster analysis based on specific combining ability to identify their heterotic grouping.

The genetic divergence created as measured by the genetic distance 0.38 from 13 initial populations (1 temperate and 12 tropical) used to generate introgressed lines, created genetic diversity that can be fully exploited in a breeding programme. Absence of a clustering pattern related to the selection environment in the study

indicated random effect of selection environment on clustering of introgressed lines. This is consistent with previous findings in the literature. Grouping based on where the inbreds were collected or selected does not always support the clustering based on molecular analysis (Pabendon et al. 2004).

3.6   Conclusion

Existing heterotic system was not disrupted by introgression of temperate germplasm into tropical elite inbred lines using a common donor parental inbred line. However 14% of the introgressed lines did not show any orientation towards the original heterotic groups. This can be attributed to recombination process that can result in recombinant lines that are different from both parents. Selection environment did not influence clustering of introgressed lines that were developed from the same base population. However allocation of introgressed lines into heterotic groups will be substantiated with phenotypic field evaluation of the introgressed lines in crosses with the testers representing each heterotic group.

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Appendices

Appendix 3.1: Profiles of each of the 20 SSR markers used

Marker Name	No. of Alleles	Allele	Marker Name	No. of Alleles	Allele	Marker Name	No. of Alleles	Allele
PHI029	4	148	PHI075	4	225	PHI308707	6	109
		153			228			111
		155			230			117
		160			239			123
PHI031	4	192	PHI084	3	155			125
		196			158			128
		225			161	PHI331888	4	130
		229	PHI102228	6	110			132
PHI056	5	237			116			133
		246			122			136
		249			124	UMC1161	6	132
		252			127			138
		255			132			144
PHI062	2	158	PHI112	3	135			145
		161			152			147
PHI065	4	136			157			150
		147				UMC1304	3	116
		152	PHI114_tailed	5	135			129
		157			139			134
PHI072	5	147			160	UMC1367	4	140
		149			163			147
		156			167			152
		160	PHI123	3	151			159
		168			154	UMC1545	6	66
nc133	3	107			156			67
		110	nc130	3	147			75
		113			150			77
					153			78
								79

Appendix 3.2: Marker, heterozygosity, motif, forward, reverse and chromosome values used to genotype 123 maize inbred lines

Marker	Heterozygosity	Motif	Forward	Reverse	Chromosome
nc130	0.901639	AGC	GCACATGAAGATCCTGCTGA	TGTGGATGACGGTGATGC	5
nc133	0.040984	GTGTC	cacgacgttgtaaaacgacAATCAAACACACACCTTGCG	GCAAGGGAATAAGGTGACGA	2
phi029	0.131148	AGCG	TTGTCTTTCTTCCTCCACAAGCAGCGAA	ATTTCCAGTTGCCACCGACGAAGAACTT	3
phi031	0.132231	GTAC	GCAACAGGTTACATGAGCTGACGA	CCAGCGTGCTGTTCCAGTAGTT	6
phi056	0.159664	CCG	cacgacgttgtaaaacgacACTTGCTTGCCTGCCGTTAC	CGCACACCACTTCCCAGAA	1
phi062	0.065574	ACG	cacgacgttgtaaaacgacCCAACCCGCTAGGCTACTTCAA	ATGCCATGCGTTCGCTCTGTATC	10
phi065	0.183333	CACTT	AGGGACAAATACGTGGAGACACAG	CGATCTGCACAAAGTGGAGTAGTC	3
phi072	0.121951	AAAC	ACCGTGCATGATTAATTTCTCCAGCCTT	GACAGCGCGCAAATGGATTGAACT	4
phi075	0.161017	CT	GGAGGAGctCACCGGCGCATAA	AAAGGTTACTGGACAAATATGC	6
phi084	0.033058	GAA	AGAAGGAATCCGATCCATCCAAGC	CACCCGTACTTGAGGAAAACCC	10
phi112	0.041322	AG	TGCCCTGCAGGTTCACATTGAGT	AGGAGTACGCTTGGATGCTCTTC	2
phi114	0.153846	GCCT	cacgacgttgtaaaacgacCCGAGACCGTCAAGACCATCAA	AGCTCCAAACGATTCTGAACTCGC	7
phi123	0.106557	AAAG	GGAGACGAGGTGCTACTTCTTCAA	TGTGGCTGAGGCTAGGAATCTC	4
phi102228	0.727273	AAGC	cacgacgttgtaaaacgacATTCCGACGCAATCAACA	TTCATCTCCTCCAGGAGCCTT	3
phi308707	0.131148	AGC	GCAACAAGATCCAGCCGAT	GTCGCCCTCATATGACCTTC	6
phi331888	0.122951	AAG	cacgacgttgtaaaacgacTTGCGCAAGTTTGTAGCTG	ACTGAACCGCATGCCAAC	5
umc1161	0.092437	GCTGGG	cacgacgttgtaaaacgacGGTACCGCTACTGCTTGTTACTGC	GCTCGCTGTTGGTAGCAAGTTTTA	8
umc1304	0.147541	TCGA	CATGCAGCTCTCCAAATTAAATCC	GCCAACTAGAACTACTGCTGCTCC	8
umc1367	0.302521	CGA	cacgacgttgtaaaacgacTGGACGATCTGCTTCTTCAGG	GAAGGCTTCTTCCTCGAGTAGGTC	10
umc1545	0.162602	AAGA	GAAAACTGCATCAACAACAAGCTG	ATTGGTTGGTTCTTGCTTCCATTA	4

* M13 tailed sequences in lower case

4 Genetic variation and Path Coefficient Analysis of introgressed inbred lines for economic traits

Abstract

Knowledge of the effects of introgressing temperate maize germplasm in tropical elite inbred lines on genetic variation and relationship between grain yield and its components is limited. In this study, the objective was to evaluate introgressed maize inbred lines for selected economic traits. Field evaluation was carried out on 122 inbred lines comprising sets of introgressed lines from three selection environments, parental inbred lines and two common checks. Genetic variation was significant (P<0.05) for all the major economic traits among inbred lines within and across sets. Heritability estimates ranged from low (0.21%) to high (91%) for stalk lodging and silking days, respectively. Comparison of means of inbred lines sets illustrated that environmental effect had influence on grain yield of introgressed lines. Grain yield and ear prolificacy performance across sets also illustrated that introgression of temperate germplasm in tropical elite inbred lines was effective. Spearman's rank correlation analysis on grain yield and ear prolificacy highlighted correlation between selection environments. Correlation among traits demonstrated that grain yield had significant (P<0.05) positive correlation with plant and ear aspects, plant height, root and stalk lodging, ear prolificacy and grain moisture content at harvest. Further, decomposing of correlation using path coefficient analysis showed significant (P<0.05), and moderate direct effects of ear prolificacy and plant height on grain yield; indicating that these traits had the highest contribution towards yield. Generally indirect effects of secondary traits on grain yield potential of inbred lines was negligible. Therefore direct selection of plant height and ear prolificacy will be emphasised during introgression of temperate germplasm in tropical elite inbred lines

Keywords: Maize, genetic variability, heritability, correlation, grain yield, grain yield components

4.1   Introduction

Maize (*Zea mays,* L) is a major staple cereal crop widely grown across environments for its productivity. In South Africa maize has a commercial value that determines social, economic and political stability of the region. South African maize industry is regarded as a net earner of foreign currency, rendering this market highly lucrative for both breeding programmes operating from tropical and temperate environments. However, tropical germplasm directly introduced into the South African environments mainly by breeding programmes operating outside the South African temperate environments has been characterized by lack of adaptability.

In the current study, the focus was on developing new maize inbred lines introgressed with genes from temperate germplasm to enhance adaptability to the South African warm temperate environments. Introgressed lines were developed through the use of a single common temperate donor inbred line as source of genes from temperate germplasm into 12 elite tropical inbred lines. Introgressed lines were selected from three distinct environments in South Africa and Zimbabwe based on important economic traits that are desirable for the South African market and are usually lacking in directly introduced germplasm namely: ear prolificacy; low grain moisture content at harvest; good plant standing ability; and high grain yield (Abadassi and Herve, 2000).

In maize breeding programmes amount of genetic variability and level of heritability determines rate of breeding progress. According to Bello et al. (2012) the success of any crop improvement programme depends upon the amount of genetic variability existing in the germplasm and the extent to which it is heritable, which sets the limit of progress that can be achieved through selection. Therefore in this study there was need to establish knowledge of the genetic variation of desired economic traits, the level of heritability among traits if increased genetic gains were to be achieved in improving desired economic traits (ear prolificacy, good standing ability, early physiological maturity and high grain yield) for the South Africa market. Literature reports significant genotypic variability and heritability among maize genotypes for various morphological traits. However, the complex nature of economical traits such as grain yield and its components in breeding programmes makes it difficult to

explore this genetic variability to achieve desired genetic gain in yield. In addition changes in environments generally affects yield mainly through its components, hence there is need to establish the relationship between yield and its components, and influence of the environment for effective selection.

Direct selection for grain yield may not be the most efficient method for crop improvement. Indirect selection for other yield related traits that are closely associated with yield and heritability estimates can be more effective (Akeel-wannows et al., 2010). Hence there is need to understand and exploit the relationships between grain yield and its components during the selection process thus ensuring grain yield improvement. According to Hefny (2011) yield components do not only directly affect selection but also indirectly by affecting other yield components in a negative or positive direction. A number of studies have reported relationship between traits using correlations and path coefficient analysis techniques. However due to inadequacy of correlation coefficients to successfully predict success of selection, several studies have explored the use of path-coefficient analysis. Path coefficient analysis has been reported in a number of studies as an efficient method for establishing correlation between grain yield and its components. Mugemangango and Kumar (2011), reports that path coefficient analysis technique establishes the exact correlation in terms of cause and effect through: identification of the direct, indirect and total (direct and indirect) casual effects. In this study Pearson's correlation and path coefficient analysis techniques were used to establish relationship of grain yield and its components.

Therefore the objective of this study was to evaluate genetic variation, heritability for selected economic traits, and to determine the relationships between traits in the new introgressed lines. Importantly, the effect of selection environment on genetic variation and mean performance should be established in order to identify suitable sites for development of introgressed lines. Environments that have high discrimination capacity would be desired to enhance breeding progress.

4.2   Materials and Methods

4.2.1   Germplasm

The experimental material comprised 123 inbred lines: 76 introgressed lines that combined temperate and tropical germplasm. These lines were selected from three distinct environments to form three sets of introgressed lines as described in Chapter 2 and were considered as test genotypes. There was also a set of 26 temperate inbred lines including the donor line that were used as a set of positive control inbred lines for the study. They were used as positive controls because they are adapted to the South African warm temperate environments. Additionally, a set of 21 tropical inbred lines was included as negative control maize inbred lines. They were considered as negative controls because they are not adapted to South African environments. The lists of these lines are indicated in Table 2.1, 2.2 and 2.3 (see Chapter 2). Therefore the new introgressed lines were evaluated in the study relative to the tropical and temperate control inbred lines.

4.2.2   Experimental design

The experimental design was an augmented alpha lattice design (Lin and Poushinsky, 1983; Scott and Miliken, 1993; Spehar, 1994). A total of 122 inbred lines (76 introgressed lines plus sets of tropical and temperate control inbred lines) were randomly assigned into six blocks, in each block 10 test entries were randomly assigned to plots within each block and two common tropical control lines (SC21 and SC19; repeated checks) were also randomly assigned in each block.

4.2.3   Field layout and agronomic management

Field layout and agronomic management was carried out at Rattray Anorld Research Station, Kadoma Research Centre, Cedara and Ukulinga Research Stations in Zimbabwe and South Africa in 2012-13 summer season. In South Africa, at Ukulinga Research Station each entry was planted to single row plots of 5m length, spaced at

0.3m in-row and 0.75m between row spacing to achieve a total plant population density of at least 44 000 plants ha^{-1}. At Cedara Research Station, single 5m row plots, in-row spacing 0.3 and inter-row 0.9m were used to achieve a plant stand of at least 37 000 plants ha^{-1}. In Zimbabwe, at Rattray Anorld Research Station and Kadoma Research Centre each entry was planted to single row plots of 10m length, space at 0.3m in-row and 1.5m between row spacing to achieve a total plant population density of at least 22 000 plants ha^{-1}. Standard cultural management practices for growing maize were carried out at all sites. Irrigation was only applied to achieve uniform establishment and also to supplement rainfall as and when necessary. Fertilizer application was done at a rate of: 120kg Nitrogen (N), 33kg Phosphorous (P), and 44kg Potassium (K) at Cedara and Ukulinga Research Stations; 145kg Nitrogen (N), 56kg Phosphorous (P), and 28kg Potassium (K) at Rattray Anorld Research Station; and 88.4 kg Nitrogen (N), 56kg Phosphorous (P), and 28kg Potassium (K) at Kadoma Research Centre.

4.2.4 Variables measured

Comprehensive data was collected at all the sites using standard procedures used at CIMMYT (1985) for the following traits: anthesis and silking days, plant and ear height, percentage stalk and root lodging, number of ears per plant (ear prolificacy), percentage grain moisture content at harvest and grain yield. The traits were measured as described in Chapter 2.

4.3 Statistical analyses

4.3.1 Analysis of variance

Data for grain yield and other agronomic traits from individual sites was analysed for variance using PROC GLM of SAS (SAS Institute Inc., 2010). Combined analysis of variance was carried out after testing for homogeneity of variance following Leven test and Welch's test using GLM procedure of SAS (SAS Institute Inc., 2010). Analysis of variance was performed using PROC GLM of SAS (SAS Institute Inc.,

2010) for combined data across sites. The means of lines were predicted for each selection environment which constituted sets. The mean of lines across the sets were also predicted. Correlation between the environments was calculated using the Spearman's rank correlation.

4.3.2 Estimation of heritability

Estimate of narrow sense heritability were performed as described by Hallauer and Miranda (1988) using the variance components analysis in SAS (SAS Institute, 2010). The heritability estimates were classified according to Robinson et al. (1949) into 3 classes; low 0-30%, medium 31-60% and >60% as high. Based on variance components narrow sense heritability was estimated as:

$$h^2 = \delta^2{}_g / (\delta^2 /re \quad + \quad \delta^2{}_{ge} /e \quad + \delta^2{}_g)$$

Where $\delta^2{}_g$ is variance of inbred lines, δ^2 is error variance, $\delta^2{}_{ge}$ is site x entry interaction variance and e is sites.

4.3.3 Estimation of Correlations

Pearson's correlation coefficients values were calculated using PROC CORR (SAS Institute, 2010).

Path coefficient analysis was used to calculate direct and indirect effects of secondary traits on grain yield using the PathSAS programme (SAS Institute, 2010) developed by Cramer et al. (1999). The path coefficient is estimated by solving sets of simultaneous equations indicating the basic relationship between correlation and path coefficients (Mugemangango and Kumar, 2011). Path coefficient direct and indirect effect values were classified into scales suggested by Lenka and Mishra (1973) namely; negligible 0.00-0.09, low 0.01-0.19, and moderate 0.20-0.29, and high 0.30-0.99. In this regard negligible values indicate non-significant contribution to grain yield potential.

$$r_{iy} = P_{iy} + r_{i1}P_{1y} + r_{i2} P_{2y} + \ldots\ldots\ldots\ldots\ldots + r_{i(i-1)} P_{iy} \; ; \; i = 1, 2, 3 \ldots\ldots n$$

Where, n is the number of independent characters; r_{1y} to r_{iy} denote coefficient of correlation between casual factors 1 to i and dependent character y, r_{i2} to $r_{(i-1)i}$ the coefficients of correlation among all possible combinations of casual factors and P_{1y} to P_{iy} denote the direct effects of character 1 to i on the character. The indirect effect of ith variable through jth variable on y the dependent variable is computed as P_{jy} x $r_{ji.}$

4.4   Results

4.4.1 Genetic variation

Combined analysis of variance for grain yield and its components for the 123 inbred lines across sites is presented in Table 4.1. Mean square values for all the traits were significantly (P<0.001) different for site effects. Control entries were significant (P<0.01) for all the traits excluding root and stalk lodging. Experimental entries were significantly (P<0.05) different for anthesis and silking days, plant and ear heights, ear prolificacy, grain moisture content at harvest and grain yield. Genotype-by-environment interaction effects were significant (P<0.05) for anthesis and silking days, root and stalk lodgings, ear prolificacy, grain moisture content at harvest and grain yield.

4.4.2 Environmental effect

Analysis of variance of grain yield and its components at individual sites showed that the four sites were able to discriminate the traits for the genotypes under study as shown in Table 4.2. Check entries at Rattray Anorld Research Station were significant (P<0.05) for the majority of the traits which included root lodging, grain moisture content at harvest, and grain yield. At Kadoma Research Centre, anthesis and silking days, ear prolificacy and grain yield were significant (P<0.05) traits for the check entries. The majority of the traits; anthesis and silking days, ear height, ear prolificacy, grain moisture content at harvest and grain yield were significant (P<0.05) at Ukulinga Research Station. At Cedara Research Station the following traits; root and stalk lodging, and ear prolificacy were significant (P<0.05). Experimental entries at Rattray

Anorld Research Station were significant (P<0.05) for anthesis and silking days, plant height, stalk lodging and grain yield. At Kadoma Research Centre silking days and grain yield were significant (P<0.05). Experimental entries were significant (P<0.05) at Ukulinga Research Station for silking days, ear height, stalk lodging, ear prolificacy, grain moisture content at harvest and grain yield. At Cedara Research Station stalk lodging and ear prolificacy were the only significant (P<0.01) traits.

Table 4. 1: Combined analyses of variances for grain yield and its components for 122 maize inbred lines across sites

Trait/Source of variation	Site	Control inbred lines	X-Experimental inbred lines (Control)	Site * Control	Site * X (Control)	MS (error)
Grain yield (t ha^{-1})	13.53***	0.41**	1.54***	0.27***	0.53***	0.05
Ear prolificacy	1.52***	4.07***	0.38***	0.34***	0.12***	0.02
Moisture content (%)	237.48***	11.63**	9.23	8.80**	4.59**	2.19
Anthesis days	6291.36***	146.19***	25.21***	25.45***	7.36***	2.79
Silking days	6381.31***	446.75***	33.04***	38.16***	6.97*	4.04
Plant height (m)	37217***	2813.17**	1528.96***	181.33	377.15	342.72
Ear height (m)	3198.56***	3839.19***	562.98***	112.51	107.06	133.93
Root lodging (%)	1960.66***	95.05	89.97**	275.50***	96.55*	41.2
Stalk lodging (%)	973.88***	29.91	52.33***	36.56	52.92***	18.78

*, **, *** indicates the data is significant at P≤ 0.05, P≤0.01, P≤ 0.001; moisture content (%), percentage grain moisture at harvest; Site, environment; Control, check entry; X(control), experimental inbred lines nested within checks; site*control, check-by-environment interaction; site*X(control), environment-by- experimental inbred lines nested within checks interaction.

Table 4. 2: Mean squares from analysis of variances for grain yield and its components for the 122 maize inbred lines at four individual sites

Trait/Source of variation	Control inbred lines				Experimental inbred lines			
	RARS	KRC	Ukulinga	Cedara	RARS	KRC	Ukulinga	Cedara
Grain yield (t ha^{-1})	0.09	0.37***	0.70*	0.03	0.95**	0.13*	1.67***	0.14
Ear prolificacy	1.69***	0.07*	1.80***	1.50**	0.16**	0.04	0.32**	0.19**
Moist content (%)	29.77	4.99	1.81**	1.26	7.41	11.57	1.27**	1.61
Anthesis days	98.86***	24.55**	97.40**	2.05	12.77***	5.07	22.68	5.86
Silking days	207.60***	173.30***	155.42**	23.57	15.268**	11.74*	21.89*	4.36
Plant height (m)	1279.63*	504.63	1340.35	403.26	478.59*	424.52	1082.85	517.66
Ear height (m)	1696.02*	501.05	780.52**	1177.3	248.06	174.19	213.63*	216.57
Root lodging (%)	20	150.5	2.75	730.56*	44.68	68.28	2.75	222
Stalk lodging (%)	1.77***	130.14	5.82	1.12***	4.56***	87.54	95.23*	12.59***

DF (check) = 2 and DF (Test lines) = 119 at all sites; *, **, *** indicates the term is significant at P≤ 0.05, P≤0.01, P≤ 0.001; RARS-Rattray Anorld Research Station; KRC-Kadoma Research Centre; Ukulinga-Ukulinga Research Station; Cedara-Cedara Research Station

Summary statistics of combined data indicated that all the data was significant (P<0.05) for the entries (Table 4.3). Anthesis and silking days had both minimum of 42 days and a maximum of 90-91 days. Plant and ear height had ranges of 0.80-2.88 m and 0.35-1.00 m, respectively. Stalk and root lodging both had minimum values of 0 % with maximum 37 %and 100 %, respectively. Variation for ear prolificacy had the smallest range 0-0.35. Large variation was also observed for the following traits: grain moisture content at harvest and grain yield.

Table 4. 3: Summary statistics of combined data for the 122 maize inbred lines across sites

Variable	Mean	Std Dev	Minimum	Maximum	R^2	CV	P value	Heritability
Grain yield (t ha^{-1})	1.30	0.99	0.00	9.92	1.00	16.60	***	0.54
Ear prolificacy	1.17	0.49	0.00	3.50	1.00	8.75	***	0.83
Grain moisture	14.84	2.90	0.00	33.30	0.98	10.57	***	0.80
Anthesis days	68.00	11.38	42.00	91.00	1.00	2.44	***	0.80
Silking days	72.00	11.43	42.00	90.00	1.00	2.93	***	0.91
Root lodging (%)	6.32	10.61	0.00	100.00	0.97	91.28	*	0.39
Stalk lodging (%)	4.54	8.31	0.00	37.00	0.99	85.21	***	0.21
Plant height (m)	1.78	36.54	0.80	2.88	0.98	11.17	***	0.70
Ear height (m)	0.78	16.26	0.35	1.50	0.97	16.23	***	0.84

*, **, *** indicates the term is significant at P≤ 0.05, P≤0.01, P≤ 0.001; Grain moisture content –grain moisture content at harvest; Std Dev-standard deviation; R^2-R-square value

4.4.3 Heritability

High heritability (h^2>0.70) was exhibited for the following traits: anthesis and silking days, grain moisture content at harvest, plant and ear heights, and ear prolificacy (Table 4.3). Moderate heritability (0.54) was estimated for grain yield. Low heritability estimates were observed for root and stalk lodging.

4.5   Comparison between breeding environments

4.5.1 Correlations between environments

Data for the two principal traits yield and ear prolificacy was used to determine the associations between test environments. Spearman's rank correlation coefficients for grain yield data between environments indicated highest positive correlation (0.81) between Cedara Research Station and Ukulinga Research Station, both in South Africa. Low correlations were noted between Rattray Anorld Research Station and Kadoma Research Centre (-0.34) in Zimbabwe, and between Cedara Research station and Kadoma Research Centre (0.17) (Table 4.4).

Table 4. 4: Environmental correlation

Spearman's rank correlation between the environments using grain yield (t ha^{-1}) data			
	RARS	KRC	Cedara	Ukulinga
RARS	1.00	-0.34	-0.01	0.01
		0.00	0.89	0.87
KRC		1.00	0.17	0.11
			<.0001	0.22
Cedara			1.00	0.81
				<0.001
Ukulinga				1.00
Spearman's rank correlation between the environments using ear prolificacy data			
	RARS	KRC	Cedera	Ukulinga
RARS	1.00	0.04	0.46	0.09
		0.65	<.0001	0.32
KRC		1.00	0.17	0.42
			0.06	<0.001
Cedara			1.00	0.26
				0.00
Ukulinga				1.00

RARS-Rattray Anorld Research Station; KRC-Kadoma Research Centre; Ukulinga-Ukulinga Research Station; Cedara-Cedara Research Station; underlined numbers-significance value *, **, *** 0.05, ≤0.01, ≤ 0.001, respectively

Spearman's rank correlation coefficients values for ear prolificacy data between environments indicated weak but significant (P<0.001) association between Cedara Research Station and Rattray Anorld Research Station (0.46), and Kadoma Research Centre and Ukulinga Research Station (0.42). Lowest association for ear prolificacy

was observed between Cedara Research Station and Ukulinga Research Station (0.26) (Table 4.4).

4.5.2 Comparison of means of lines derived in different environments

Results of least significant mean data for grain yield and its components of inbred line sets across sites were significant (P<0.05) for: silking days, ear prolificacy, grain moisture content at harvest and grain yield (Table 4.5). Inbred line sets (introgression and controls) were different for grain yield potential. Grain moisture content at harvest data indicated no differences among the inbred line sets with the only difference observed between recipient inbred lines (tropical elite inbred lines) and donor parental inbred line (temperate inbred lines). Ear prolificacy showed difference among introgressed lines bred at Rattray Anorld Research Station and the other two sets of introgressed lines from Kadoma Research Centre and Ukulinga Research Station. Control inbred line sets were different for ear prolificacy in tropical and temperate germplasm. Least significant means values for silking days showed difference between donor line and tropical inbred lines, while introgressed lines were not different. The results in Table 4.5 indicates that the donor parent (temperate) was superior for the principal traits, grain yield, ear prolificacy, grain moisture content at harvest and silking days. The tropical set of inbred lines was generally inferior to both the donor lines and the set of temperate lines for the economic traits. Although recipient lines (founder parents) displayed higher grain yield than the introgression sets, they were inferior to their progenies with respect to ear prolificacy grain moisture content at harvest and silking days.

Table 4. 5: Least significant means for grain yield and its components of maize inbred lines sets across sites

Set	GY	EPP	MC	AD	SD	PH	EH	RL	SL
Introgressed lines ex-KRC	1.33b	1.47d	13.52ab	66.53	66.02a	44.37	18.39	4.61	4.85
Introgressed lines ex-RARS	1.43c	1.41c	13.88ab	67.76	67.83ab	49.43	19.1	4.42	4.17
Introgressed lines ex-Ukulinga	1.11a	1.49d	13.75ab	68.47	68.38ab	48.3	20.28	4.8	3.88
Controls									
A-recipient lines	1.77d	1.11b	15.06b	68.67	69.52ab	45.77	20.21	4.04	4.64
B-donor line	2.59e	1.75e	13.01a	66.21	65.44a	49.92	17.21	4.95	7.82
C-tropical lines	1.28b	1.06a	14.60ab	70.79	72.63b	47.83	23.04	3.43	2.69
D-temperate lines	1.47c	1.75e	13.77ab	69.80	69.53ab	41.41	18.64	3.75	3.77
Trial mean	1.57	1.43	13.94	68.32	68.48	46.72	19.55	4.29	4.54
CV (%)	68.78	30.89	20.58	8.69	16.63	129.12	124.38	121.54	148.26
LSD$_{(0.05)}$	0.06	0.03	1.66	0.86	6.58	6.50	4.17	1.92	2.03
Pr>F	***	***	*	NS	*	NS	NS	NS	NS

Ex-KRC, introgressed lines bred at Kadoma Research Centre; Ex-RARS, introgressed lines bred at Rattray Anorld Research Station; Ex-Ukulinga, Introgressed lines bred at Ukulinga Research Station, A-recipient lines, tropical lines crossed to donor line; B-donor line, common donor parent; C-tropical lines, tropical inbred lines; D-temperate lines, temperate inbred lines; *, **, *** indicates the term is significant at P≤ 0.05, P≤0.01, P≤ 0.001; NS, not significant

4.6 Correlations between traits

4.6.1 Correlation analysis

Significant (P<0.05) positive and negative correlations were observed between primary and secondary traits (Table 4.6). The main primary trait, grain yield had positive correlation with plant and ear height, root and stalk lodging, ear prolificacy and grain moisture content at harvest; but negative correlation with anthesis days and silking days. Ear prolificacy had positive correlation with plant and ear height, and anthesis days; while negative correlation was observed with root and stalk lodging. Secondary traits had positive correlation observed between traits namely: anthesis days and silking days, ear height and flowering days (anthesis and silking days), root lodging and anthesis days, ear height and plant height, root lodging and flowering days, and grain moisture content at harvest and flowering days. Negative correlation was also detected among secondary traits namely; plant height and flowering days, stalk lodging and flowering days, stalk lodging and plant height, stalk lodging and ear height.

4.6.2 Path coefficient analysis

In this study, the correlations coefficients of secondary traits on grain yield were further partitioned into direct and indirect effects using path coefficient analysis. Plant height and ear prolificacy showed significant (P<0.05) direct effect on grain yield (Table 4.7). In this study categorizes of path coefficient values suggested by Lenka and Mishra (1973) were used as: negligible 0.00-0.09, low 0.01-0.19, and moderate 0.20-0.29, and high 0.30-0.99. Significant (P<0.05) and moderate positive direct effect values for grain yield were observed on plant height and ear prolificacy (Table 4.8). Plant height had a moderate positive direct effect (0.27) on grain yield and it also illustrated negligible positive indirect effect via the following traits; silking days (0.04), stalk lodging (0.03), ear prolificacy (0.01) and grain moisture content at harvest (0.03). Plant height also illustrated negligible negative indirect effect via: anthesis days (-0.01) and ear height (-0.03). Ear prolificacy showed moderate direct effect

(0.24) on grain yield and it also illustrated negligible positive indirect effect via; silking days (0.06), and plant height (0.01), while negligible negative indirect effect were observed via; anthesis days (-0.01), stalk lodging (-0.01) and grain moisture content at harvest.

Table 4. 6: Correlation coefficients for grain yield and its components of maize inbred lines

	Anthesis days	Silking days	Plant height	Ear height	Root Lodging	Stalk lodging	Ear prolificacy	Grain moisture	Grain yield
Anthesis days	1	0.98***	-0.13***	0.21***	0.23***	-0.32***	0.10*	0.48***	-0.0.7*
Silking days		1	-0.16***	0.21***	0.19***	-0.35***	0.04	0.5***	-0.16***
Plant height			1	0.6***	-0.03	-0.15***	0.32***	-0.01	0.38***
Ear height				1	0.03	-0.11*	0.17***	0.36***	0.30***
Root Lodging					1	0.25***	-0.12***	0.03***	0.14***
Stalk lodging						1	-0.27***	-0.17***	0.19***
Ear prolificacy							1	0.05	0.26***
Grain moisture								1	0.16*
Grain yield									1

R^2 =0.66; n=525;*, **, ***-Significant at 0.5, 0.01, 0.001, respectively; grain moisture-grain moisture content at harvest

Table 4. 7: Parameter estimates for direct effects based on regression

Trait	Parameter estimate	Standard Error	t Value	Pr > |t|
Intercept	-0.064	0.059	-1.070	0.287
Anthesis days	0.150	0.146	1.020	0.309
Silking days	-0.274	0.154	-1.780	0.079
Plant height	0.266	0.109	2.440	0.017*
Ear height	-0.042	0.109	-0.380	0.703
Root lodging	0.000	0.070	0.000	0.996
Stalk lodging	0.145	0.078	1.850	0.068
Ear prolificacy	0.240	0.090	2.670	0.009**
Grain moisture	0.096	0.097	0.980	0.329

*, **, ***-Significant at 0.5, 0.001, respectively

Table 4. 8: Direct and indirect effects of secondary traits on grain yield of maize inbred lines

	anthesis days	silking days	plant height	ear height	root lodging	stalk lodging	ear prolificacy	grain moisture	Total correlation with GYD
anthesis days	**0.15**	-0.24	-0.02	-0.01	0.00	-0.04	-0.02	0.04	-0.07
silking days	0.13	**-0.27**	-0.03	-0.01	0.00	-0.04	-0.05	0.04	-0.17
plant height	-0.01	0.04	**0.27**	-0.03	0.00	0.03	0.01	0.03	0.38*
ear height	0.02	-0.04	0.21	**-0.04**	0.00	0.03	0.00	0.05	0.30
root lodging	-0.02	0.03	0.07	-0.01	**0.00**	0.05	0.00	0.02	0.14
stalk lodging	-0.04	0.08	0.06	-0.01	0.00	**0.14**	-0.02	0.00	0.19
ear prolificacy	-0.01	0.06	0.01	0.00	0.00	-0.01	**0.24**	-0.01	0.26***
Grain moisture	0.07	-0.12	0.09	-0.02	0.00	0.00	-0.02	**0.10**	0.16

R^2 =0.66; n=525; Bold font and underlined for direct effects; *, **, ***-Significant at 0.5, 0.1, 0.01, respectively; grain moisture-grain moisture content at harvest

4.7   Discussion

4.7.1   Genetic variability among inbred lines

The amount of genetic variability in maize breeding population sets the limit of genetic gain that can be attained in improving traits of economic importance. Significant variation was observed among inbred lines within and among sets for all the traits which was an indication that genetic variation for the traits under study was present. Bello et al. (2012) reports that the success of any crop improvement programme depends upon the amount of genetic variability existing in the germplasm, which sets the limit of progress that can be achieved through selection. Therefore this indicates that traits that illustrated genetic variation in this current study can be exploited for improvement. However breeding progress might be slow as genotype-environment interaction was also observed to be significant. A number of studies have also reported genetic variation for economic traits such as: anthesis and silking days (Hefny, 2011; Akeel wannows et al., 2010); plant and ear height (Kage et al., 2013; Bello et al., 2011); root and stalk lodging (Prasanna, 2012); ear prolificacy (Kesomkeaw et al. 2009; Golam et al., 2011); grain moisture content at harvest (Filipenco et al., 2013); and grain yield (Bello et al., 2011).

Combined analysis of variance was significant (P<0.001) due to site effect for all the traits. Therefore the sites were different from each other and provided contrasting environments for testing inbred lines' performance. Check entries were significant for all the traits under study excluding percentage root and stalk lodging. This showed that the check entries gave a wide spectrum of traits to compare against the introgressed lines; an indication that they were appropriate checks which can be used for similar studies in the future. However, in future studies, there is also the need to select checks that accommodate all the traits under study. Genotype-by-environment interaction was significant (P<0.05) for the majority of the traits. This illustrates that phenotypic selection of economic traits under study was influenced by environmental effects, an indication that there may be slow breeding progress, because G x E compromises heritability.

4.7.2 Genetic variability across inbred lines sets

Least significant mean values for grain yield and its components of inbred line sets across sites were significant (P<0.05). Grain yield illustrated that there was genetic variation among the introgressed lines bred from different environments. This was an indication that there are environmental effects for grain yield potential of introgressed lines bred at different environments. Grain yield for the control entries were significant Illustrating genetic variation for grain yield potential of temperate and tropical germplasm used in this study. Inbred line sets (introgressed and controls) were different for grain yield potential. Grain moisture content at harvest indicated no differences among the inbred line sets. This can be attributed to the common donor parental inbred line that was used during introgression. In addition, recipient tropical inbred lines used during introgression came from an established tropical breeding programme; and breeders are known to recycle breeding material during trait improvement. Therefore this may account for no difference observed for grain moisture content at harvest among inbred lines within and across sets. Differences observed between recipient inbred lines and donor parental inbred line for grain moisture content at harvest can be attributed to difference in germplasm backgrounds. Temperate germplasm has low grain moisture content at harvest relative to tropical germplasm (Abadassi and Herve, 2000). Similar results have been reported by Tarter et al. (2004) of high grain moisture content at harvest in tropical maize germplasm relative to temperate maize germplasm. However it is clear that further introgression of temperate germplasm in the introgressed lines will be required to boost variation for grain moisture content at harvest. This is one of the principal traits that will confer adaptation of inbred lines in temperate environments

Difference noted in ear prolificacy among introgressed lines bred at Rattray Anorld Research Station and sets of introgressed lines bred at Kadoma Research Centre and Ukulinga Research Station indicates the influence of breeding environments in discriminating ear prolificacy among inbred lines (Table 4.4). Hence Rattray Anorld Research Station was a unique environment for selecting for ear prolificacy during breeding. Introgression of temperate germplasm for ear prolificacy was effective as indicated by general difference in ear prolificacy among the introgressed lines.

Control inbred line sets were different for ear prolificacy in tropical and temperate germplasm. This can be attributed to differences in germplasm background. According to Brathwaite and Brathwaite (2002) and Kesomkeaw et al. (2009) genetic diversity and high heritability for ear prolificacy is more pronounced in temperate germplasm relative to tropical germplasm. Silking days indicated differences between the donor line and tropical inbred lines, indicating effect of germplasm background on flowering. Introgressed lines were not different for silking days which can be attributed to common donor parent line used during introgression. Therefore further introgression of the lines using different donor parental inbred lines will be pursued to obtain amble genetic variation for silking days in introgressed lines.

4.7.3 Environmental correlation

Correlation between sites was also observed to be significant (P<0.001) between Rattray Anorld Research Station and Ukulinga Research Station, Kadoma Research Centre and Cedara Research, and Kadoma Research Centre and Ukulinga Research Station, using grain yield data (Table 4.5). However, only Kadoma Research Centre and Ukulinga Research Station had a high correlation coefficient value of 0.80, indicating only one of the sites could be recommended for utilization during breeding and trial evaluation as they have the same discriminating effect. However this result is in sharp contrast with known records as Kadoma Research Centre is situated in tropical environments of Zimbabwe; while Ukulinga Research Station is a temperate environment in South Africa. The remaining sites Rattray Anorld Research Station and Ukulinga Research Station; and Kadoma Research Centre and Cedara Research Station had weak correlation coefficient values (0.29) and (0.17), respectively. The sites offer contrasting environmental effects hence they can be used as different selection environments. Similar trend was also observed for these environments using ear prolificacy trait.

Analysis of variance for grain yield and its components at the four individual sites illustrated difference in discriminating effect of desirable economic traits for the genotypes at each site used. Significant differences for anthesis days and silking

days for all the environments except Cedara Research Station indicates differences in genetic variation to flowering and also influence of environmental factors such as day length, temperature effect, and growing degree units per season during flowering in maize (Abadassi and Herve, 2000; Edmeades et al., 2000; Xu et al., 2009). Ukulinga Research Station illustrated significant differences (P<0.01) for ear height for entries an indication that the environment can effectively discriminate inbred lines for ear height hence it can be used for phenotypic selection of ear height in introgressed lines. Significant difference (P<0.05) for root lodging were observed at Cedara Research Station only which can be attributed to excessive wind storms that are experienced at this site, qualifying it as the best site to screen introgressed lines for standing ability.

Number of ears per plant was observed to be significantly different (P<0.05) at all the sites except for Kadoma Research Centre environment. Therefore these environments can discriminate ear prolificacy in inbred lines under study and can effectively be utilized for phenotypic selection in future studies. Lack of ear prolificacy at Kadoma Research Centre can be attributed to the drought stress associated with below normal rains that were received during the growing season (Table 2.4) (see Chapter 2). Edmeades et al. (1997) also reports that ear prolificacy is a secondary trait in maize production which is affected by barrenness under drought stress. This is contrary to Varga et al. (2004) who reported increased number of ears per plant under stress environment. Percentage grain moisture content at harvest was observed to be significant (P<0.001) at Rattray Anorld Research Station only, which can be attributed to early harvesting that was carried out at this site. Therefore in future studies, time of harvesting of trials should be standardized across sites based on physiological maturity. Grain yield data also illustrated that all the sites were able to discriminate inbred lines and showed genetic variation for grain yield which enables selection. Therefore phenotypic selection can be carried out on introgressed lines at these sites based on grain yield potential. Breeding programmes prefer inbred lines with outstanding grain yield potential in seed production.

4.7.4 Heritability

High narrow sense heritability estimates were observed for the following traits: anthesis and silking days, percentage grain moisture content at harvest, plant and ear height and ear prolificacy. This illustrates that these traits can be successfully selected for using phenotypic selection during breeding. Based on the high heritability estimates, these traits can also be used as part of the selection index for the improvement of introgressed lines in future projects. Similar results have been reported in a number of studies for the following traits: anthesis and silking days (Beyene, 2005; Sumathi et al., 2005); plant and ear height (Smalley et al., 2003; Akeel-wannows et al., 2010; Bello et al., 2011). Moderate heritability was detected for grain yield. This indicates that grain yield is a complex trait that is strongly influenced by environment during selection thus slow progress is expected during selection. Contrasting reports on the magnitude of heritability for grain yield has also been reported: low heritability (Sumathi et al., 2005; Iqbal, 2009) and high heritability (Beyene, 2005; Akeel-wannows et al., 2010). Differences in the heritability values among researchers can be attributed to differences in genetic materials that were used as well as environments used during the studies. Low heritability estimates for root and stalk lodging were detected. This indicates that the traits may be influenced by environmental factors that mask genetic effects during selection. Therefore they are difficult to directly select for in breeding for the introgressed lines. Further breeding gains for these traits can be obtained by increasing genetic variance, and improving quality of experiments to minimise error during assessment.

4.7.5 Correlation among traits

A number of traits in maize have complex inheritance hence they are difficult to directly select for in breeding programmes, therefore there is need to indirectly select these traits using other closely related traits. In this study, Pearson's correlation coefficient and path coefficient values were used to establish relationships among grain yield and its components.

4.7.5.1   Correlation analysis

The traits under study illustrated that there was significant (P<0.05), positive and negative correlation between traits. Grain yield had positive correlation with plant and ear height, root and stalk lodging, ear prolificacy and grain moisture content. This shows that grain yield is a complex trait that is affected by both yield and growth aspects of the plant. Varga et al. (2004) reports that ear prolificacy is a yield component that has a direct effect on grain yield. In this current study, growth aspects were observed to have positive effect on grain yield. This reveals that indirect selection of growth aspects; plant and ear height, root and stalk lodging, and grain moisture content at harvest may result in improved grain yield. Similar results have been reported of positive correlation of grain yield with ear prolificacy (Varga et al., 2004) and plant height (Iqbal, 2010). In contrast, anthesis and silking days had negative correlation with grain yield. This may illustrate that these traits have an inverse relationship with grain yield; selection for anthesis and silking days may lead to low grain yield. Ear prolificacy illustrated positive correlation with plant and ear height, and anthesis days. This indicates that selection of these plant attributes will result in an increased ear prolificacy. Secondary traits that demonstrated positive correlation between each other may suggest that these traits can be indirectly selected for each other thus ensure parallel improvement of these traits. An improvement in one of the traits has a direct effect on the corresponding trait during selection. However, negative correlation was also observed between secondary traits an indication that these traits have an inverse relationship. An increase in one trait will lead to a decline in the corresponding trait, therefore there has to be a compromise during selection when breeding for both traits.

4.7.5.2   Path coefficient analysis

This study revealed that there was significant (P<0.05), moderate and positive direct effects of plant height and ear prolificacy on grain yield, an indication that these traits had the highest contribution towards grain yield. It is evident that an increase in ear

prolificacy and plant height can result in an increase grain yield. This may reveal that plant height and ear prolificacy maybe given a high selection preference during breeding. Beside positive direct effect on grain yield, plant height also revealed negligible positive indirect effect via silking days, stalk lodging, ear prolificacy and grain moisture content at harvest. Thus by selecting for plant height one would also be selecting for these traits. Similarly, when selecting for ear prolificacy indirect selection for silking days and plant height will also be achieved.

4.8   Conclusion

The study revealed genetic variation among inbred lines within sets and among sets for all the economic traits evaluated. Heritability estimates were detected varying from low (21%) to high (91%) for stalk lodging and silking days, respectively. Comparison of means of introgressed lines bred from different environments illustrated that selection environments had an effect on grain yield of inbred lines. Difference and ear prolificacy performance of the new progeny lines across sets illustrated that introgression of temperate germplasm into tropical elite inbred lines was effective. Spearman's rank correlations on grain yield and ear prolificacy showed correlation between environments. This indicated that Kadoma Research Centre would be suitable environment to evaluate germplasm to be deployed in the South Africa environments. Correlation analysis showed that grain yield had positive correlations with plant and ear height, root and stalks lodging, and also yields components such as ear prolificacy and grain moisture content at harvest. Further breakdown of the correlations by path analysis revealed that there was significant (P<0.05), and moderate direct effect of plant height and ear prolificacy on grain yield. This indicates that these are the most important traits contributing towards grain yield. The indirect effects of secondary traits on grain yield were generally small to negligible. In sum it indicated that plant height and ear prolificacy must be emphasised during the introgression strategy to enhance adaptation of tropical germplasm in South African warm temperate environments.

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5 Assessment of genetic gains from introgression of temperate genes into tropical elite maize inbred lines: I. Performance *per se*

Abstract

There is very limited information on genetic gains and improvement on maize inbred line performance *per se* that has been attained through introgression of temperate germplasm into tropical maize germplasm to enhance adaptability in target environments. The objective of this study was to determine the genetic gains for grain yield and its components achieved by introgression of temperate germplasm into tropical elite maize inbred lines. A total of 122 maize inbred lines comprising of 76 introgressed lines, 21 tropical inbred lines (negative controls) and 26 temperate inbred lines (positive controls) were evaluated in an alpha lattice augmented design at four sites. The strategy was effective and successful to enhance grain yield, in particular ear prolificacy. Positive genetic gains were realised for grain yield (5%) and ear prolificacy (46%) relative to the population and mean of checks. Selection for plant and ear height, root and stalk lodging had gains ranging from 2% to 11%. However traits such as anthesis and silking days and grain moisture content at harvest had low gains. Introgressed line performance *per se* was impressive because new lines with potential for commercial production were obtained. Inbred line 71-DMLF7_88 showed combined early physiological maturity, high ear prolificacy and yield potential that were at par with the mean of the temperate checks. In conclusion, introgression of temperate germplasm into tropical elite inbred lines was effective to improve adaptation in warm temperate environments.

Keywords: inbred lines, genetic gain, grain yield, grain yield components, performance *per se*

5.1   Introduction

Success of maize breeding programmes competing for the lucrative South African maize seed market is partially dependent on a cost effective seed production system. However, the majority of the programmes struggle with production of certified and parent seed at a price that will make the end product profitable. According to Morris (1998) the undeniable successes of competitive seed companies is dependent on developing high quality seed, and distribution of seed at attractive prices. Increasing competition in the South African seed industries has resulted in seed quality and producibility assuming an increasingly important role. Therefore breeding programmes should focus on increasing genetic gains of desired economic traits that enhance quality and producibility of the seed. Inbred line performance is critical because it determines producibility of the hybrids in which the inbred line is involved.

Singh (2006) reported that selection during inbred line development should be effective in improving the performance of inbred lines themselves, which is an important factor in hybrid seed production. In maize breeding programmes it should however be noted that general inbred line performance *per se* is not as important as identification of inbred lines that produce outstanding hybrid performance in hybrid combinations (*inter se* performance). Therefore assessment of genetic gains for introgression of temperate germplasm in tropical elite inbred lines performance *inter se* is reported in Chapter 6. In this current Chapter, focus is on assessing the genetic gains of an introgression breeding programme that aimed at introgressing temperate germplasm into tropical elite inbred lines to enhance performance *per se* of the inbred lines in the South African temperate environments. The end product will be a single cross hybrid; the cost of seed production will depend on the yield potential of the inbred parents.

A population comprising; tropical recipient inbred lines (founder parents), temperate parental inbred lines (control), donor parental inbred line (temperate founder parent) and the new progeny introgressed lines was evaluated. However, the introgressed lines were bred at three distinct environments which might have altered the genetic variation of the lines. According to Abbott et al. (2012) environmental effects often

lead to differential response of materials of different regions. Therefore the study focus was on assessment of the effects of introgression of temperate germplasm into tropical elite inbred lines on grain yield potential and its enabling components. It was also prudent in this study to establish introgressed lines *per se* performance as a measure of the effectiveness of the introgression strategy in improving desired economic traits that are highly desirable for the South African market namely; good plant standing ability, reduced rank growth, early flowering, and low grain moisture content at harvest and high yield potential.

A number of studies have reported the importance of genetic gain in maize breeding programmes with particular emphasis on grain yield increase. Duvick et al. (2004), reports that an estimated 51% of the total increase in maize yield in Iowa has been due to genetic gain. Ci et al. (2011) attributes recent genetic improvements for grain yield of maize hybrids and open pollinated varieties grown in China to increase in genetic gain in stress tolerance of these varieties. Badu-Aparaku (2013) reports an increase in genetic gain in maize cultivars under *striga*-infestation that is credited to improvement in ear aspect, lodging resistance, plant height and increase in number of ears per plant, increased days to anthesis, increased ear and plant height. Despite the extensive documentation of genetic gains for grain yield and its related phenotypic attributes in maize hybrid and open pollinated varieties, there is very limited information on genetic gains that were achieved by introgression of temperate germplasm into tropical elite inbred lines, especially in South Africa warm temperate environments.

Poor adaptability of tropical germplasm in temperate environments is mainly attributed to lack of economical traits that are required in this environment. Therefore the focus of the study was to estimate genetic gains of traits that enhance adaptability; grain yield and ear prolificacy were the main primary traits; plant aspects that contribute towards good standing ability, plant and ear heights, root and stalk lodging; and early physiological maturity which is related to early flowering and low grain moisture content at harvest constituted the secondary traits.

The objectives of the current study were to assess; genetic gains for grain yield and its components, as a basis of gaining information for response to selection; and to establish introgressed lines *per se* performance for adaptation to South Africa warm temperate environments. Overall the information obtained in the study would form the basis for judging whether the introgression strategy was effective for breeding new generation of inbred lines that are adapted to the South African warm temperate environment.

5.2   Materials and Methods

5.2.1   Germplasm

The experimental material comprised 122 inbred lines: 76 introgressed lines that combined temperate and tropical germplasm. These lines were developed as described in Chapter 2 and were considered as test genotypes. There was also a set of 26 temperate inbred lines including the donor line that were used as positive controls because they are adapted to the South African warm temperate environments. Additionally, a set of 21 tropical inbred lines was included as negative controls, because they are not adapted to South African environments. The lists of these lines are indicated in Table 2.1, 2.2 and 2.3 (see Chapter 2).

5.2.2   Experimental design

The experimental design was an augmented alpha lattice design (Lin and Poushinsky, 1983; Scott and Miliken, 1993; Spehar, 1994) with two common check inbred lines (SC19 and SC21) as described in Chapter 4. The experiment was replicated over four sites: Rattray Anorld Research, Kadoma Research Centre, Cedara and Ukulinga Research Stations as described in Table 2.4 (see Chapter 2).

5.2.3   Field layout and agronomic management

In South Africa, at Ukulinga Research Station each entry was planted to single row plots of 5m length, spaced at 0.3m in-row and 0.75m between row spacing to achieve a total plant population density of at least 44 000 plants ha^{-1}. At Cedara Research Station, single 5m row-plots, in-row spacing 0.3m and between row 0.9m were used to achieve a plant stand of at least 37 000 plants ha^{-1}. In Zimbabwe, at Rattray Anorld Research Station, and Kadoma Research Centre each entry was planted to single row plots of 10m length, spaced at 0.3m in-row and 1.5m between row spacing to achieve a total plant population density of at least 22 000 plants ha^{-1}. Standard cultural management practices for growing maize were carried out at all the sites. Irrigation was only applied to achieve uniform establishment and also to supplement rainfall as and when necessary. Fertilizer application was done at a rate of: 120kg Nitrogen (N), 33kg Phosphorous (P), and 44kg Potassium (K) at Cedara and Ukulinga Research Stations; 145kg Nitrogen (N), 56kg Phosphorous (P), and 28kg Potassium (K) at Rattray Anorld Research Station; and 88.4 kg Nitrogen (N), 56kg Phosphorous (P), and 28kg Potassium (K) at Kadoma Research Centre.

5.2.4 Variables measured

Comprehensive data was collected at all sites using standard procedures used at CIMMYT (1985) for the following traits: anthesis and silking days, plant and ear heights, percentage stalk and root lodgings, number of ears per plant, percentage grain moisture content at harvest and grain yield. The traits were measured as described in Chapter 2.

5.3   Statistical analyses

5.3.1 Analysis of variance

Data for grain yield and other agronomic traits from individual sites and combined sites was subjected to general analysis of variance (ANOVA) using PROC GLM of SAS (SAS Institute Inc., 2010) with genotype as the main factor. Before a combined analysis of variance was carried out, test for homogeneity of variance following Levene test and Welch's test was conducted using GLM procedure of SAS (SAS Institute Inc., 2010).

The linear statistical model for the combined data:

$$Y_{ijk} = \mu + B_i + C_j + X_k(C) + Eijk$$

Where: *Y_{ijk} = observed inbred response; μ = overall trial mean; B_i = effect of the ith block; I =1 …6; C_j = effect of the jth inbred control; j = 1.2; X_k (C) = effect of the experimental inbred within checks; k = 1… 120; E_{ijk} = random experimental error. The block effects were treated as random while the inbred main effects were considered fixed.*

5.3.2 Estimation of means and genetic parameters

The data measurements were used to compute and estimate genetic parameter at 10% selection intensity for grain yield and its components as follows:

1. Mean of selected set of inbred lines (MS), that is the best 10% introgressed lines.
2. Mean of population (MP)
3. Mean of better check (MBC)
4. Mean of checks (MCS)

The two check inbred lines used in the study are parents of an elite hybrid (SC633) which has been widely grown in South Africa, and therefore for a heterotic pattern which requires improvement.

5. Realised genetic gain 1 (%) (RG1 %) was calculated relative to the population mean of all 122 inbred lines, using the method suggested by Singh and Chaudhary (2004) and Souza et al. (2009) using the equation:

$$RG\ 1\ (\%) = ((MS\text{-}MP)/MP) * 100$$

6. Realised genetic gain 2 (%) (RG2 %) was calculated relative to the mean of the better check inbred line, using a modified method suggested by Singh and Chaudhary (2004) using the equation:

$$RG\ 2\ (\%) = ((MS\text{-}MBC)/MBC) * 100$$

7. Realised genetic gain 3 (%) (RG3 %) was calculated relative to the mean of the two repeated check inbred lines, using a modified method suggested by Singh and Chaudhary (2004) using the equation:

$$RG\ 3\ (\%) = ((MS\text{-}MC)/MC) * 100$$

8. Narrow sense heritability (%) h^2; was estimated using the variance ratio (Hallauer and Miranda, 1988). The variance component analysis was performed using the PROC Varcomp procedure in SAS (SAS Institute, 2010). Therefore heritability was estimated using the equation:

$$h^2 = \delta^2{}_g / (\delta^2 /re \quad + \quad \delta^2{}_{ge} /e \quad + \delta^2{}_g)$$

Where $\delta^2{}_g$ is variance entry, δ^2 is variance error, $\delta^2{}_{ge}$ is variance site x entry interaction and e is the number of sites.

9. Coefficient of genotypic variation percentage (CGV %), was calculated according to Singh and Chaudhary (2004); Souza et al. (2009) and Al-Tabbal (2012) using the equation:

$$(CGV\ \%) = (\sqrt{(\delta^2{}_g)}/x) * 100$$

Where: $\delta^2{}_g =$ genotypic variance, $X =$ mean of selected inbred lines.

10. Coefficient of genotypic variation as a function of coefficient of variance (GCV/CV), was calculated according to Souza et al. (2009) using the equation:

$$CGV/CV$$

Where: CV was obtained using PROC GLM of SAS (SAS Institute Inc., 2010)

11. Genetic gain was calculated using the method suggested by Singh and Chaudhary (2006) and Al-Tabbal (2012) using the equation:

$$GG = i * \sigma_p * h^2$$

Where GG: genetic gain; i: standardized selection differential = 1.76 at 10 % selection intensity; σ_p: is the phenotypic standard deviation; h^2: heritability in narrow sense.

12. Genetic gain percentage was calculated using the method suggested by Souza et al. (2009) using the equation:

$$GG\ (\%) = (i * \sigma_p * h^2) * 100$$

5.4   Results

5.4.1 Realised genetic gain for inbred lines

Estimate of realised genetic gain of grain yield and its components of the selected introgressed lines highlighted a general gain relative to the population mean and mean of the inbred checks. The main primary traits; grain yield and ear prolificacy showed that introgression strategy resulted in a desired positive gain of 5% to 46%, respectively across sites relative to the mean of the population and the mean of checks (Table 5.1). A similar trend was observed at all individual sites (Table 5.2 and 5.3).

The plant aspects; plant and ear height, root and stalk lodging had significant desired gains of 2% to 11% relative to the mean of inbred checks across sites (Table 5.1). However, the selected introgressed lines had higher gains in the negative direction relative to the population mean for all the plant aspects. Stalk lodging was inferior relative to the check inbreds. In addition, exceptionally large gains were observed in the negative direction relative to the mean of checks at Cedara and Ukulinga Research Station (Table 5.3). The attributes of early physiological maturity; anthesis and silking days, and grain moisture content at harvest had low gains achieved relative to the population mean and the mean of inbred checks (Table 5.1). A comparable trend was also noted at individual sites.

5.4.2 Realised genetic gain: Grain yield of individual inbred lines

Using grain yield as the main selection trait, summary of grain yield and its components at 10% selection intensity indicated that all the introgressed lines out-yielded tropical inbred line checks across sites (Table 5.4). Most importantly the top ten selected lines had better performance relative to mean of temperate inbred lines. However the selected lines were out yielded by the donor parent inbred line. Individual sites had varying trends observed with Kadoma Research Centre (tropical environment) which had the top three selected introgressed lines outperforming all the inbred checks (Table 5.4), while at Ukulinga Research Station (temperate environment) all the selected introgressed lines had high yield potential relative to all the inbred checks except the donor line. Regrettably there was no improvement for ear prolificacy across sites (Table 5.4). However significant improvements were noted at individual sites namely Rattray Anorld Research Station (tropical environments) (Table 5.5), Cedara and Ukulinga Research Stations (temperate environments) (Table 5.6).

 Early physiological maturity attributes as reflected by anthesis and silking days, and grain moisture at harvest showed that there was desirable gain relative to the mean of temperate lines and the donor parent line across sites (Table 5.4). At least three of the top high yielding introgressed lines also combined these attributes with high yield

potential. A comparable trend was also observed at individual sites (Table 5.5 and 5.6). Plant aspects such as plant and ear heights, root and stalk lodging that contribute towards good standing ability showed negligible improvement relative to the mean of temperate lines across sites (Table 5.4). Comparable trend was also observed for all the traits at individual sites except stalk lodging at Rattray Anorld Research Station that had significant (P≤0.05) improvement noted.

Table 5. 1: Estimates of realised and predicted genetic gain of grain yield and its components of top performing inbred lines selected at 10% selection intensity across sites

	Across site genetic gain														
	Realised genetic gain							Predicted genetic gain							
Traits	MS	MP	MBC	MCS	RG 1 (%)	RG 2 (%)	RG 3 (%)	δ$_g$	CGV (%)	h^2 (%)	CV	CGV/CV	St dev	GG	GG (%)
AD	67.19	68.28	70.88	70.79	-1.58	-5.19	-5.08	6.87	3.90	81.46	2.44	0.02	2.07	2.97	4.35
SD	66.59	68.20	72.46	72.63	-2.36	-8.10	-8.31	11.88	5.18	91.21	2.93	0.02	1.62	2.60	3.81
PH	1.74	1.65	1.78	1.77	5.30	-2.04	-1.51	0.02	8.74	70.20	11.14	0.01	0.10	0.13	7.90
EH	0.73	0.70	0.78	0.83	4.09	-6.34	-11.35	0.01	15.34	83.51	16.22	0.01	0.06	0.09	12.54
RL	12.04	10.44	13.13	9.02	15.36	-8.27	33.44	6.99	21.96	38.67	82.93	0.00	4.84	3.29	31.56
SL	7.58	6.98	5.00	5.71	8.60	51.56	32.76	1.71	17.27	21.11	65.10	0.00	2.59	0.96	13.79
EPP	1.55	1.48	1.17	1.06	4.67	31.69	46.20	0.10	20.80	83.16	8.71	0.02	0.17	0.25	16.85
MC	13.81	13.98	14.84	14.60	-1.20	-6.94	-5.43	4.60	15.53	79.80	10.53	0.01	0.76	1.07	7.63
GYD	1.87	1.42	1.30	1.28	31.45	43.61	45.90	0.17	21.78	54.04	16.62	0.01	0.28	0.27	18.74

MS -mean of sampled population; MP- mean of total population; MBC- mean of better check; MCS -mean of all checks; RG 1 (%) - percentage realised gain 1; RG 2 (%) - percentage realised gain 2; RG 3 (%) –percentage realised gain 3; δ$_g$ –genetic variance; CGV -coefficient of genotypic variation; h^2 (%) – percentage heritability; CV-coefficient of variance; St dev- standard deviation; GG-genetic gain; GG (%) –percentage genetic gain; AD, anthesis days; SD, silking days; PH, plant height (m); EH, ear height (m); SL, percentage stalk lodging; RL, percentage root lodging; EPP, number of ears per plant (ear prolificacy); MC, percentage grain moisture content at harvest; GYD, grain yield (t ha^{-1}).

Table 5. 2: Estimates of realised and predicted genetic gain of grain yield and its components of top performing inbred lines selected at 10% selection intensity at individual sites

	Realised genetic gain							Predicted genetic gain							
					Rattray Arnold Research Station										
Traits	MS	MP	MBC	MCS	RG 1 (%)	RG 2 (%)	RG 3 (%)	δ_g	CGV (%)	h^2 (%)	CV	CGV/CV	St dev	GG	GG (%)
AD	70.58	78.83	79.67	78.83	-10.47	-11.4	-10.47	15.84	5.64	100	0.49	0.12	3.43	6.04	7.66
SD	71.12	78.67	81.67	78.67	-9.59	-12.91	-9.59	33.49	8.14	100	1.27	0.06	2.92	5.14	6.53
PH	1.58	1.58	1.65	1.58	0.22	-4.17	0.22	0.02	8.97	42.35	5.64	0.02	0.13	0.10	6.14
EH	0.80	0.86	0.97	0.86	-7.02	-17.24	-7.02	0.03	20.05	96.07	12.97	0.02	0.11	0.19	21.62
SL	1.86	1.93	0.67	0.67	-3.32	179.17	179.17	1.08	55.93	6.06	190.89	0.00	1.54	0.16	8.54
RL	1.00	0.78	0.00	0.67	29.03	.	50.00	0.16	40.28	3.37	98.8	0.00	0.60	0.04	4.59
EPP	1.78	1.25	0.92	1.25	42.38	94.09	42.38	0.28	29.57	100.00	8.06	0.04	0.29	0.51	40.84
MC	16.00	18.22	19.62	18.22	-12.19	-18.46	-12.19	4.31	12.98	75.57	14.4	0.01	2.06	2.74	15.04
GYD	2.10	1.43	1.48	1.43	47.66	41.85	47.66	0.80	42.46	47.23	13.26	0.03	0.42	0.35	24.68
					Kadoma Research Centre										
AD	67.29	67.27	70.00	69.17	0.04	-3.87	-2.71	3.68	12.19	70.39	1.71	0.07	1.34	1.66	2.47
SD	66.96	66.8	79.5	74.58	0.23	-15.78	-10.22	28.36	12.21	100.00	1.96	0.06	2.11	3.71	5.56
PH	1.41	1.30	1.38	1.40	8.33	1.81	0.60	0.01	80.96	14.30	11.87	0.07	0.11	0.03	2.13
EH	0.69	0.61	0.65	0.70	12.26	6.09	-0.90	0.01	113.66	37.09	17.06	0.07	0.08	0.05	8.50
SL	1.85	1.89	1.83	1.58	-2.51	0.76	16.67	0.15	74.52	9.60	66.53	0.01	0.68	0.11	6.06
RL	2.17	2.33	1.67	1.50	-6.92	30.00	44.44	0.59	70.42	21.85	32.24	0.02	0.95	0.37	15.69
EPP	1.05	1.07	0.94	0.97	-1.62	12.46	8.78	0.01	98.23	25.21	12.97	0.08	0.11	0.05	4.56
MC	12.72	13.35	14.02	14.26	-4.69	-9.22	-10.75	1.96	28.71	75.59	15.30	0.02	1.56	2.08	15.55
GYD	2.09	1.62	1.72	1.69	29.15	21.35	24.11	0.06	60.85	46.84	16.38	0.04	0.20	0.16	10.18

MS -mean of selected population; MP- mean of total population; MBC- mean of better check; MCS -mean of all checks; RG 1 (%) - percentage realised gain 1; RG 2 (%)- percentage realised gain 2; RG 3 (%)–percentage realised gain 3; δ_g –genetic variance; CGV -coefficient of genotypic variation; h^2 (%) – percentage heritability; CV-coefficient of variance; St dev- standard deviation; GG-genetic gain; GG (%) –percentage genetic gain; AD, anthesis days; SD, silking days; PH, plant height (cm); EH, ear height (cm); SL, percentage stalk lodging; RL, percentage root lodging; EPP, number of ears per plant (ear prolificacy); MC, percentage grain moisture content at harvest; GYD, grain yield (t ha^{-1}).

Table 5. 3: Estimates of realised and predicted genetic gain of grain yield and its components of top performing inbred lines selected at 10% selection intensity at individual sites

	Realised genetic gain							Predicted genetic gain							
						Cedara Research Station									
Traits	MS	MP	MBC	MCS	RG 1 (%)	RG 2 (%)	RG 3 (%)	δ_g	CGV (%)	h^2 (%)	CV	CGV/CV	St dev	GG	GG (%)
AD	81.33	82.05	81.50	82.08	-0.88	-0.20	-0.91	0.91	1.17	14.35	1.58	0.01	1.04	0.26	0.32
SD	79.92	81.13	81.50	82.75	-1.50	-1.94	-3.42	3.15	2.22	66.33	2.95	0.01	1.36	1.59	1.96
PH	2.08	1.92	2.04	1.97	8.31	2.07	5.59	0.01	3.78	12.36	5.17	0.01	0.56	0.12	6.35
EH	0.90	0.75	0.88	0.92	19.93	3.10	-2.21	0.02	17.29	100.00	17.61	0.01	0.11	0.19	25.74
RL	3.38	1.61	2.33	3.25	109.45	44.64	3.85	2.94	50.77	63.74	81.85	0.01	1.05	1.18	73.10
SL	0.00	0.07	0.00	0.00	-100.00	.	.	0.08	.	25.04	0.00	.	0.09	0.04	59.01
EPP	1.77	1.69	1.53	1.25	4.95	16.31	41.71	0.25	28.15	100.00	7.87	0.04	0.23	0.40	23.95
MC	14.70	14.09	14.63	14.57	4.30	0.43	0.89	6.14	16.86	81.24	5.22	0.03	0.75	1.07	7.61
GYD	3.83	2.41	2.62	2.52	59.27	46.37	51.98	0.69	21.66	33.40	16.09	0.01	0.58	0.34	14.16
						Ukulinga Research Station									
AD	48.12	48.48	54.33	55.08	-0.74	-11.43	-12.63	15.50	8.18	66.84	4.34	0.02	2.93	3.45	7.11
SD	47.37	47.53	54.33	56.08	-0.33	-12.81	-15.53	25.69	10.70	100.00	0.99	0.11	2.76	4.86	10.22
PH	2.10	2.12	2.03	2.04	-1.30	3.46	2.95	0.01	4.16	6.96	18.21	0.00	0.18	0.02	1.04
EH	0.67	0.68	0.72	0.79	-1.73	-6.50	-14.44	0.01	15.99	53.40	9.32	0.02	0.54	0.51	74.26
SL	3.46	2.95	1.00	1.00	17.23	245.83	245.83	0.17	11.98	3.66	106.09	0.00	0.88	0.06	1.92
RL	0.79	0.75	0.17	0.25	5.56	375.00	216.67	0.07	32.24	12.19	265.82	0.00	0.21	0.05	6.01
EPP	1.75	1.82	1.07	0.97	-3.75	63.73	80.64	0.31	31.90	96.44	12.13	0.03	0.34	0.58	31.76
MC	12.19	12.26	11.10	11.38	-0.56	9.83	7.18	0.40	5.16	100.00	2.26	0.02	0.27	0.48	3.88
GY	3.51	3.64	1.73	1.56	-3.61	103.04	124.13	0.18	12.16	11.51	12.40	0.01	0.67	0.14	3.73

MS -mean of sampled population; MP- mean of total population; MBC- mean of better check; MCS -mean of all checks; RG 1 - percentage realised gain 1; RG 2 - percentage realised gain 2; RG 3 –percentage realised gain 3; δ_g –genetic variance; CGV -coefficient of genotypic variation; h^2 (%) – percentage heritability; CV-coefficient of variance; St dev- standard deviation; GG-genetic gain; GG (%) –percentage genetic gain; AD, anthesis days; SD, silking days; PH, plant height (cm); EH, ear height (cm); SL, percentage stalk lodging; RL, percentage root lodging; EPP, number of ears per plant (ear prolificacy); MC, percentage grain moisture content at harvest; GYD, grain yield (t ha^{-1}).

5.4.3 Predicted genetic gain

Estimates of predicted genetic gain for grain yield and ear prolificacy were 19% and 17%, respectively across sites (Table 5.1). Comparable gains were observed at all the sites excluding Rattray Anorld Research Station (Table 5.2) that had higher gains. Unfortunately gains observed for plant attributes for good standing ability were in the undesirable direction across sites as well as at individual sites. Anthesis and silking days and grain moisture content at harvest are important traits that had low gain observed across sites (Table 5.1). Similar trend was also noted at individual sites (Table 5.2 and 5.3)

Negligible genetic variation for plant and ear height, ear prolificacy and grain yield were observed across sites (Table 5.1). Genetic variation for anthesis and silking days, root and stalk lodging, and grain moisture content at harvest ranged from 1.71 to 11.88. Coefficient of genotypic variation estimates ranged from 3.90% to 21.96% for anthesis days and root lodging, respectively across sties (Table 5.1). High heritability estimates were observed for the majority of the traits except root and stalk lodging, 38.67% and 21.11%, respectively. High coefficient of variation estimates was observed for root and stalk lodging across sites (Table 5.1). Similar trend was also observed at individual sites (Table 5.2 and 5.3). However, an exception was observed for coefficient of genotypic variation estimates for root and stalk lodging that had high values at Rattray Anorld Research Station; and also root lodging at Cedara Research Station.

Table 5. 4: **Summary of the top 12 performing inbred lines using grain yield as main selection trait at 10% selection intensity across sites**

Entry	GY	EPP	MC	AD	SD	PH	EH	RL	SL
7	2.35	1.7	14.34	66.10	68.28	2.09	0.92	7.43	6.18
32	2.31	1.83	13.09	66.39	64.75	1.79	0.77	3.99	5.15
50	2.26	1.65	13.36	66.23	64.94	1.60	0.61	5.02	3.71
36	2.13	1.58	14.98	66.98	65.81	1.73	0.72	12.02	5.46
41	1.93	1.52	14.68	67.42	68.28	2.06	1.04	9.71	9.08
47	1.89	1.74	15.14	69.32	68.63	1.72	0.61	1.55	4.49
28	1.87	1.15	14.47	66.10	65.41	1.98	0.79	7.36	6.21
48	1.79	1.34	13.22	65.64	65.63	1.63	0.71	13.49	4.90
14	1.78	1.76	13.59	67.35	64.97	1.71	0.7	6.46	10.36
60	1.78	1.63	14.22	66.10	70.34	1.68	0.7	39.3	4.36
16	1.64	1.22	12.27	68.76	67.09	1.52	0.69	34.24	26.15
17	1.31	1.43	12.39	67.76	66.66	1.38	0.53	3.9	4.90
Mean of selected lines	1.87	1.55	13.81	67.19	66.59	1.74	0.73	12.04	7.58
Mean of population	1.42	1.48	13.98	68.28	68.2	1.65	0.7	10.44	6.98
Check 1 (tropical line)	1.26	0.94	14.37	70.71	72.79	1.76	0.87	4.92	6.42
check 2 (tropical line)	1.3	1.17	14.84	70.88	72.46	1.78	0.78	13.13	5.00
Mean of checks (tropical lines)	1.28	1.06	14.6	70.79	72.63	1.77	0.83	9.02	5.71
Mean of temperate lines	1.48	1.75	13.75	69.6	69.17	1.61	0.68	6.3	5.92
Donor parent	2.54	1.81	12.85	66.48	65.44	1.88	0.76	10.15	4.77
Mean of recipient parents	1.33	1.06	13.42	67.24	68.91	1.58	0.61	5.95	5.48
LSD$_{(0.05)}$	0.02	0.01	0.13	0.15	0.18	0.02	0.06	0.77	0.39
CV (%)	16.62	8.71	10.53	2.44	2.93	11.14	16.22	82.93	65.10
Pr >F	***	***	***	***	***	***	***	*	***

AD, anthesis days; SD, silking days; PH, plant height (m); EH, ear height (m); SL, percentage stalk lodging; RL, percentage root lodging; EPP, number of ears per plant (ear prolificacy); MC, percentage grain moisture content at harvest; GYD, grain yield (t ha^{-1})

Table 5. 5: Summary of top 12 performing inbred lines using grain yield as main selection trait at 10 % selection intensity at individual sites

	Rattray Arnold Research Station									Kadoma Research Centre									
Entry	GYD	EPP	MC	AD	SD	PH	EH	SL	RL	Entry	GYD	EPP	MC	AD	SD	PH	EH	RL	SL
7	4.13	2.19	17.07	69.00	66.00	1.98	1.13	4.67	1.33	7	2.32	0.97	13.71	64.00	63.00	1.78	0.77	2.58	6.50
33	2.43	1.94	16.02	70.00	70.00	1.61	0.88	0.67	2.67	63	2.18	0.97	9.26	68.00	67.00	1.68	0.87	2.58	4.50
30	2.38	2.23	11.87	72.00	70.00	1.46	0.63	0.67	0.67	41	2.16	0.97	12.46	67.00	67.00	1.63	0.92	2.58	2.50
42	2.33	1.17	20.27	70.00	73.00	1.61	0.88	0.67	0.67	52	2.13	0.97	13.61	69.00	74.00	1.58	0.67	1.58	0.50
48	1.93	1.44	14.67	76.00	74.00	1.33	0.88	0.67	1.33	57	2.11	1.68	11.41	69.00	69.00	1.25	0.62	1.08	0.00
10	1.83	1.35	14.57	63.00	65.00	1.41	0.73	0.67	0.67	40	2.11	0.97	11.81	68.00	70.00	1.13	0.47	0.58	0.50
28	1.83	1.36	18.67	69.00	71.00	1.96	0.88	0.67	0.67	32	2.06	1.28	11.91	65.00	64.00	1.43	0.62	1.58	3.50
50	1.83	2.28	13.37	73.00	70.00	1.38	0.68	0.67	1.33	50	2.06	0.97	12.51	68.00	67.00	1.18	0.42	4.58	0.50
32	1.73	1.98	17.37	74.00	75.00	1.51	0.88	1.33	0.67	55	2.05	0.97	10.21	66.00	64.00	1.18	0.57	0.42	1.50
13	1.63	1.48	17.17	65.00	68.00	1.76	0.83	0.67	0.67	22	2.00	0.97	12.71	67.00	64.00	1.28	0.72	0.42	1.50
8	1.63	2.01	13.82	70.00	71.00	1.48	0.58	1.33	0.67	34	1.97	0.97	15.46	67.00	66.00	1.50	0.87	1.58	1.50
60	1.63	1.94	17.12	75.00	79.00	1.48	0.68	9.67	0.67	43	1.97	0.97	17.66	69.00	69.00	1.33	0.75	2.58	3.00
Mean of selected lines	2.10	1.78	16.00	71.00	71.00	1.58	0.80	1.86	1.00		2.12	1.07	11.96	67.00	67.00	1.41	0.67	1.80	2.15
Mean of population	1.43	1.25	18.22	79.00	79.00	1.58	0.86	1.93	0.78		2.13	1.13	12.07	67.00	67.00	1.45	0.64	2.23	2.30
Check 1 (tropical line)	1.37	1.58	16.82	78.00	76.00	1.51	0.75	0.67	1.33		1.65	1.00	14.50	68.00	70.00	1.42	0.74	1.33	1.33
check 2 (tropical line)	1.48	0.92	19.62	80.00	82.00	1.65	0.97	0.67	0.00		1.72	0.94	14.02	70.00	80.00	1.38	0.65	1.83	1.67
Mean of checks (tropical)	1.43	1.25	18.22	79.00	79.00	1.58	0.86	0.67	0.67		1.69	0.97	14.26	69.00	75.00	1.40	0.70	1.58	1.50
Mean of temperate lines	2.04	1.82	16.61	76.00	77.00	1.50	0.75	2.35	1.00		1.50	1.11	12.59	68.00	67.00	1.20	0.56	2.05	2.22
Donor parent	2.23	1.94	12.37	70.00	68.00	1.73	0.88	4.67	1.33		1.46	1.50	12.16	68.00	66.00	1.43	0.65	3.58	5.00
Mean of recipient parents	1.53	1.15	18.27	73.00	76.00	1.51	0.78	3.17	1.39		1.60	0.81	9.74	67.00	67.00	1.27	0.50	1.69	2.25
LSD$_{(0.05)}$	0.06	0.03	0.67	0.10	0.27	0.02	0.04	0.51	0.41		1.14	1.02	1.11	0.37	0.40	0.97	1.17	2.31	1.61
CV (%)	13.26	8.06	14.40	0.49	1.27	5.64	12.97	190.89	98.80		16.38	12.97	15.30	1.71	1.96	11.87	17.06	66.53	32.24
Pr>F	NS	***	NS	***	***	*	*	***	NS		***	***	NS	**	**	NS	NS	NS	NS

AD, anthesis days; SD, silking days; PH, plant height (m); EH, ear height (m); SL, percentage stalk lodging; RL, percentage root lodging; EPP, number of ears per plant (ear prolificacy); MC, percentage grain moisture content at harvest; GYD, grain yield (t ha^{-1}) ; NS, not significant

Table 5. 6: Summary of top 12 performing inbred lines using grain yield as main selection trait at 10 % selection intensity at individual sites

	Cedara Research Station									Ukulinga Research Station									
Entry	GYD	EPP	MC	AD	SD	PH	EH	RL	SL	Entry	GYD	EPP	MC	AD	SD	PH	EH	SL	RL
7	4.69	2.00	14.17	81	80	2.28	1.17	0.75	0.00	36	4.64	1.97	12.53	48.00	48.00	1.97	0.68	0.50	0.25
63	4.09	1.46	14.97	82	80	2.34	0.83	4.75	0.00	32	4.22	2.25	11.88	50.00	48.00	2.27	0.80	1.00	0.25
41	4.07	1.8	15.67	81	83	2.43	1.42	3.75	0.00	47	3.87	2.44	11.98	53.00	46.00	2.14	0.57	1.00	0.25
52	3.96	1.54	15.02	82	80	2.4	0.98	4.25	0.00	50	3.86	1.77	12.28	48.00	56.00	1.90	0.62	1.00	0.25
40	3.90	1.59	13.57	81	80	2.23	1.11	2.75	0.00	60	3.82	1.92	12.13	52.00	50.00	1.97	0.47	0.50	1.25
57	3.85	2.63	14.37	81	79	2.09	1.03	1.75	0.00	9	3.31	1.79	12.28	45.00	46.00	2.42	0.80	0.50	0.25
50	3.82	1.67	14.32	81	79	2.05	0.56	5.75	0.00	41	3.29	1.59	12.03	48.00	47.00	2.34	0.90	5.50	3.25
55	3.65	1.82	13.17	82	80	1.69	0.66	0.25	0.00	69	3.21	1.69	12.13	47.00	46.00	2.06	0.63	15.50	0.25
32	3.61	1.78	12.52	80	79	1.94	0.83	0.75	0.00	14	3.14	1.72	12.38	48.00	46.00	1.95	0.68	0.50	1.25
43	3.48	1.74	16.32	82	81	1.54	0.49	6.75	0.00	28	2.98	1.01	13.03	47.00	46.00	2.23	0.71	3.50	0.25
22	3.47	1.72	14.27	81	78	2.12	0.91	3.75	0.00	4	2.95	1.10	11.63	41.00	42.00	1.93	0.55	10.50	1.25
34	3.39	1.56	18.02	82	80	1.81	0.83	5.25	0.00	56	2.74	1.71	12.08	52.00	52.00	1.99	0.68	1.50	0.75
Mean of selected lines	3.83	1.77	14.7	81	80	2.08	0.9	3.38	0.00		3.51	1.75	12.19	48.00	47.00	2.10	0.67	3.46	0.79
Mean of population	2.41	1.69	14.09	82	81	1.92	0.75	1.61	0.07		1.88	1.55	11.87	51.00	51.00	1.88	0.69	1.58	0.56
Check 1 (tropical line)	2.43	0.98	14.5	83	84	1.9	0.97	4.17	0.00		1.40	0.87	11.65	56.00	58.00	2.05	0.85	1.00	0.33
check 2 (tropical line)	2.62	1.53	14.63	82	82	2.04	0.88	2.33	0.00		1.73	1.07	11.10	54.00	54.00	2.03	0.72	1.00	0.17
Mean of checks (tropical)	2.52	1.25	14.57	82	83	1.97	0.92	3.25	0.00		1.56	0.97	11.38	55.00	56.00	2.04	0.79	1.00	0.25
Mean of temperate lines	2.12	2.08	13.88	82	81	1.87	0.73	1.13	0.13		1.76	2.01	12.04	54.00	53.00	1.83	0.69	0.78	0.55
Donor parent	1.77	2.03	14.52	82	84	1.84	0.62	0.25	0.00		4.99	1.99	11.88	50.00	48.00	2.37	0.88	2.00	0.25
Mean of recipient parents	2.27	1.24	13.99	81	80	1.89	0.63	0.75	0.00		1.22	0.74	11.75	49.00	52.00	1.68	0.56	1.67	0.50
LSD$_{(0.05)}$	0.44	0.37	1.06	2.56	2.55	0.39	0.25	0.36	0.07		0.09	0.07	0.11	0.89	0.83	0.14	0.03	0.51	0.28
CV (%)	16.09	7.87	5.22	1.58	2.95	5.17	17.61	81.85	0.00		12.40	12.13	2.26	4.34	0.99	18.21	9.32	106.09	265.80
Pr>F	NS	**	NS	NS	NS	*	NS	*	***		*	**	**	**	*	NS	**	NS	NS

AD, anthesis days; SD, silking days; PH, plant height (m); EH, ear height (m); SL, percentage stalk lodging; RL, percentage root lodging; EPP, ears per plant (ear prolificacy); MC, percentage grain moisture content at harvest; GYD, grain yield (t ha^{-1}); NS, not significant

5.4.4 Realised genetic gain: Performance of individual inbred lines

Ear prolificacy is an important primary trait that has direct positive correlation with grain yield (see chapter 4). Brathwaite and Brathwaite (2002), report that yield increase in improved maize cultivars is associated with increase in ear prolificacy. The top performing inbred lines selected using ear prolificacy as the selection criteria are shown in Table 5.7. The selected inbred lines demonstrated significant (P<0.05) high ear prolificacy relative to the mean of temperate inbred lines across sites. Most importantly, the top five selected introgressed lines exhibited significantly (P≤0.05) higher ear prolificacy relative to the donor inbred line. A comparable trend was observed at the individual sites excluding Rattray Anorld Research Station which was not significant (Table 5.8 and 5.9). The top selections for ear prolificacy had six introgressed lines that also exhibited high grain yield potential relative to the donor inbred line across sites (Table 5.7). Individual site data showed the same trend for improved ear prolificacy combined with high yield potential relative to the check inbreds (Table 5.8 and 5.9)

However there was concern that the selected introgressed lines had inferior performance for early physiological maturity attributes; anthesis and silking days, and grain moisture content at harvest relative to the mean of inbred checks across sites (Table 5.7). However selected introgressed line 71-DMLF7_88 combined early physiological maturity, high ear prolificacy and almost similar yield potential relative.to the mean of the temperate lines. Individual sites also reflected inferior performance for early physiological maturity attributes (Table 5.8 and 5.9). Plant aspects for good plant structure such as plant and ear heights, stalk and root lodging indicated that the top three selected introgressed lines (57-DMLF730, 58-DMLF_33 and 21-KRC_35) combined all the desired attributes for better standing ability relative to the mean of inbred checks (Table 5.7). Plant aspects for standing ability were not significant for the majority of the sites, excluding Rattray Arnold Research Station (Table 5.8) that had attributes for better standing ability, ear height and root lodging relative to the mean of the temperate inbred lines and the donor inbred line.

Table 5. 7: Summary of top 12 performing inbred lines using ear prolificacy as main selection trait across site

Entry	EPP	GY	MC	AD	SD	PH	EH	RL	SL
57	1.96	1.59	13.95	70.01	68.66	1.64	0.76	2.93	3.80
58	1.93	0.95	12.85	71.67	70.97	1.26	0.47	3.65	3.02
21	1.91	1.01	13.04	67.73	66.25	1.56	0.57	0.71	4.61
56	1.86	1.50	14.55	68.23	67.69	1.64	0.69	4.05	3.86
32	1.83	2.31	13.09	66.39	64.75	1.79	0.77	3.99	5.15
71	1.81	1.06	12.75	68.12	67.50	1.62	0.65	14.83	4.05
31	1.78	1.16	12.30	67.76	67.34	1.74	0.79	22.96	4.49
30	1.76	1.53	13.63	67.98	67.50	1.79	0.75	12.58	4.30
14	1.76	1.78	13.59	67.35	64.97	1.71	0.70	6.46	10.36
11	1.75	1.55	13.96	66.76	65.78	1.70	0.73	9.02	4.05
47	1.74	1.89	15.14	69.32	68.63	1.72	0.61	1.55	4.49
2	1.74	1.17	15.74	65.14	66.00	1.71	0.64	18.05	3.24
Mean of selected lines	1.82	1.46	13.72	68.03	67.14	1.66	0.68	8.40	4.62
Mean of population	1.48	1.42	13.98	68.28	68.20	1.65	0.70	10.44	6.98
Check 1 (Tropical lines)	0.94	1.26	14.37	70.71	72.79	1.76	0.87	4.92	6.42
Check 2 (Tropical lines)	1.17	1.30	14.84	70.88	72.46	1.78	0.78	13.13	5.00
Mean of Checks	1.06	1.28	14.60	70.79	72.63	1.77	0.83	9.02	5.71
Mean of temperate lines	1.75	1.48	13.75	69.60	69.17	1.61	0.68	6.30	5.92
Donor parent	1.81	2.54	12.85	66.48	65.44	1.88	0.76	10.15	4.77
Mean of recipient parents	1.06	1.33	13.42	67.24	68.91	1.58	0.61	5.95	5.48
LSD(0.05)	0.01	0.02	0.13	0.15	0.18	0.02	0.06	0.77	0.39
CV	8.71	16.62	10.53	2.44	2.93	11.14	16.22	82.93	65.10
Pr> F	***	***	***	***	***	***	***	***	***

Selection intensity 10%; AD, anthesis days; SD, silking days; PH, plant height (m); EH, ear height (m); SL, percentage stalk lodging; RL, percentage root lodging; EPP, ears per plant (ear prolificacy); MC, percentage moisture content at harvest; GYD, grain yield (t ha^{-1}).

Table 5. 8: Individual sites summary of grain yield and its components for the top 12 performing inbred lines using ear prolificacy as main selection trait

	Rattray Anorld Research Station									Kadoma Research Centre								
Entry	EPP	GYD	MC	AD	SD	PH	EH	SL	RL	Entry	EPP	GY	MC	AD	SD	PH	EH	SL	RL
50	2.80	1.03	16.47	72.83	77.83	1.36	0.83	1.33	5.67	57	1.68	2.11	11.41	69.17	69.33	1.25	0.62	0.00	1.08
30	2.37	1.08	12.47	74.83	72.83	1.56	0.83	4.67	0.67	56	1.68	1.08	14.01	70.17	68.33	1.30	0.62	1.00	1.08
7	2.28	1.83	13.37	72.83	70.33	1.38	0.68	0.67	1.33	68	1.66	1.84	11.11	69.17	69.33	1.20	0.47	2.00	2.08
74	2.23	2.38	11.87	71.83	69.83	1.46	0.63	0.67	0.67	66	1.59	1.76	15.36	65.67	63.83	1.35	0.77	3.50	2.58
59	2.19	4.13	17.07	68.83	66.33	1.98	1.13	4.67	1.33	48	1.56	1.71	11.81	67.17	65.33	1.40	0.57	5.00	5.08
71	2.18	0.83	17.42	75.33	76.83	1.43	0.78	2.67	0.67	2	1.54	1.58	14.11	68.17	66.33	1.50	0.52	5.00	5.08
14	2.16	0.93	12.47	70.83	73.33	0.83	0.33	0.67	1.33	21	1.49	1.70	11.91	65.67	62.83	1.53	0.62	2.50	1.58
8	2.16	0.73	14.22	71.33	70.83	1.48	0.43	24.67	0.67	19	1.40	1.71	11.91	67.17	66.33	1.20	0.47	0.00	1.08
99	2.05	1.23	15.82	77.33	74.83	1.53	0.73	1.33	5.67	33	1.39	1.86	16.06	62.67	61.83	1.30	0.92	6.50	3.58
97	2.01	1.63	13.82	70.33	70.83	1.48	0.58	1.33	0.67	46	1.36	1.56	16.11	69.17	69.33	1.30	0.67	1.00	1.08
96	1.98	1.73	17.37	73.83	74.83	1.51	0.88	1.33	0.67	32	1.28	2.06	11.91	65.17	63.83	1.43	0.62	3.50	1.58
102	1.94	1.63	17.12	75.33	78.83	1.48	0.68	9.67	0.67	11	1.28	1.62	13.21	66.17	65.83	1.38	0.62	0.50	0.58
Mean of selected lines	2.20	1.59	14.95	72.96	73.12	1.46	0.70	4.47	1.67		1.49	1.71	13.24	67.13	66.04	1.34	0.62	2.54	2.21
Mean of population	0.86	0.78	1.93	1.25	1.43	18.22	78.83	78.67	1.58		1.07	1.62	13.35	67.27	66.80	1.30	0.61	2.33	1.89
Check 1 (Tropical line)	0.75	1.33	0.67	1.58	1.37	16.82	78.00	75.67	1.51		1.00	1.65	14.50	68.33	69.67	1.42	0.74	1.33	1.33
Check 2 (Tropical line)	0.97	0.00	0.67	0.92	1.48	19.62	79.67	81.67	1.65		0.94	1.72	14.02	70.00	79.50	1.38	0.65	1.67	1.83
Mean of Checks	0.86	0.67	0.67	1.25	1.43	18.22	78.83	78.67	1.58		0.97	1.69	14.26	69.17	74.58	1.40	0.70	1.50	1.58
Mean of temperate lines	0.75	1.00	2.35	1.82	2.04	16.61	76.35	76.77	1.50		1.11	1.50	12.59	67.67	67.47	1.20	0.56	2.22	2.05
Donor parent	0.88	1.33	4.67	1.94	2.23	12.37	69.83	68.33	1.73		1.50	1.46	12.16	68.17	65.83	1.43	0.65	5.00	3.58
Mean of recipient parents	0.78	1.39	3.17	1.15	1.53	18.27	73.08	75.75	1.51		0.81	1.60	9.74	67.17	67.08	1.27	0.50	2.25	1.69
LSD(0.05)	0.04	0.41	0.51	0.03	0.06	0.67	0.10	0.27	0.02		1.02	1.14	1.11	0.37	0.40	0.97	1.17	1.61	2.31
CV	12.97	98.80	190.89	8.06	13.26	14.40	0.49	1.27	5.64		12.97	16.38	15.30	1.71	1.96	11.87	17.06	32.24	66.53
Pr> (0.05)	NS	*	NS	**	**	NS	***	**	*		**	**	NS	NS	*	NS	NS	*	NS

Selection intensity 10%; AD, anthesis days; SD, silking days; PH, plant height (cm); EH, ear height (cm); SL, percentage stalk lodging; RL, percentage root lodging; EPP, ears per plant (ear prolificacy); MC, percentage grain moisture content at harvest; GYD, grain yield (t ha^{-1}); NS, not significant.

Table 5. 9: Summary of grain yield and its components for top 12 performing inbred lines using ear prolificacy as main selection trait at 10 % selection intensity at individual sites

	Cedara Research Station									Ukulinga Research Station									
Entry	EPP	GYD	MC	AD	SD	PH	EH	RL	SL	Entry	EPP	GY	MC	AD	SD	PH	EH	SL	RL
57	2.63	3.85	14.37	81.08	79.25	2.09	1.03	1.75	0.00	58.00	3.37	0.82	12.18	58.08	52.58	1.50	0.57	0.50	0.25
56	2.38	0.82	13.72	82.08	84.25	1.79	0.62	0.25	0.00	47.00	2.44	3.87	11.98	52.58	45.58	2.14	0.57	1.00	0.25
75	2.27	1.50	14.07	82.08	78.75	1.87	1.01	0.25	0.00	25.00	2.41	2.15	11.28	50.58	49.08	2.40	1.02	4.50	0.75
47	2.26	1.34	14.32	82.08	83.25	2.04	0.60	0.25	0.00	46.00	2.36	2.33	12.23	49.08	51.58	1.70	0.53	0.50	0.25
21	2.23	2.55	14.07	81.08	77.25	1.84	0.62	0.75	0.00	32.00	2.25	4.22	11.88	49.58	47.58	2.27	0.80	1.00	0.25
71	2.08	1.78	13.92	#	#	2.23	0.84	1.75	0.00	2.00	2.21	2.04	11.88	44.58	45.58	2.12	0.65	0.50	0.25
66	2.05	2.71	13.27	82.08	79.25	1.49	0.71	0.25	0.00	57.00	2.18	2.45	12.18	56.58	58.58	1.88	0.58	1.00	0.25
67	2.02	0.38	15.42	82.08	85.25	1.75	0.63	1.75	0.00	22.00	2.09	1.54	11.83	56.08	55.58	1.77	0.42	0.50	0.25
64	2.02	3.09	11.82	81.08	79.75	1.85	0.88	1.25	0.00	6.00	2.06	2.26	11.98	43.58	43.08	2.28	0.88	1.50	0.75
7	2.00	4.69	14.17	81.08	79.75	2.28	1.17	0.75	0.00	71.00	2.06	2.04	12.28	44.58	43.58	2.10	0.73	0.50	1.25
30	1.99	1.83	14.32	81.08	82.25	2.34	0.77	2.75	0.00	21.00	2.01	1.01	12.08	50.58	51.08	1.86	0.58	0.50	0.75
13	1.96	3.17	12.92	85.08	80.75	1.63	0.64	1.75	0.00	36.00	1.97	4.64	12.53	48.08	47.58	1.97	0.68	0.50	0.25
Mean of selected lines	2.16	2.31	13.86	81.90	80.89	1.94	0.79	1.13	0.00		2.29	2.45	12.02	50.33	49.29	2.00	0.66	1.04	0.46
Mean of population	1.69	2.41	14.09	82.05	81.13	1.92	0.75	1.61	0.07		1.75	3.51	12.19	48.12	47.37	2.10	0.67	3.46	0.79
Check 1 (Tropical line)	0.98	2.43	14.50	82.67	84.00	1.90	0.97	4.17	0.00		0.87	1.40	11.65	55.83	57.83	2.05	0.85	1.00	0.33
Check 2 (Tropical line)	1.53	2.62	14.63	81.50	81.50	2.04	0.88	2.33	0.00		1.07	1.73	11.10	54.33	54.33	2.03	0.72	1.00	0.17
Mean of Checks	1.25	2.52	14.57	82.08	82.75	1.97	0.92	3.25	0.00		0.97	1.56	11.38	55.08	56.08	2.04	0.79	1.00	0.25
Mean of temperate lines	2.08	2.12	13.88	82.37	81.38	1.87	0.73	1.13	0.13		2.01	1.76	12.04	54.47	53.15	1.83	0.69	0.78	0.55
Donor parent	2.03	1.77	14.52	82.08	84.25	1.84	0.62	0.25	0.00		1.99	4.99	11.88	49.58	47.58	2.37	0.88	2.00	0.25
Mean of recipient parents	1.24	2.27	13.99	80.68	80.35	1.89	0.63	0.75	0.00		0.73	1.22	11.75	48.92	51.67	1.68	0.56	1.67	0.50
LSD(0.05)	0.31	0.43	1.06	2.54	2.54	0.39	0.23	0.24	0.00		0.07	0.09	0.11	0.89	0.83	0.14	0.03	0.51	0.28
CV	7.87	16.09	5.22	1.58	2.95	5.17	17.61	81.85	0.00		12.13	12.40	2.26	4.34	0.99	18.21	9.32	106.09	265.82
Pr> F	**	**	**	*	*	NS	*	NS	NS		**	***	**	*	*	NS	*	NS	NS

AD, anthesis days; SD, silking days; PH, plant height (cm); EH, ear height (cm); SL, percentage stalk lodging; RL, percentage root lodging; EPP, ears per plant (ear prolificacy); MC, percentage grain moisture content at harvest; GYD, grain yield (t ha^{-1}); #, missing data; NS, not significant.

5.5   Discussion

5.5.1 Realised genetic gain

Gains achieved for selection of introgressed lines at 10% selection intensity for grain yield and its components illustrated that introgression of temperate germplasm in tropical germplasm was an effective strategy for improving desired economic traits. Realised genetic gain measures the actual genetic gain achieved in trait improvement (Weng et al., 2008). The main primary traits; grain yield and ear prolificacy demonstrated positive desired gains relative to the population mean and mean of checks. Genetic gain achieved can be attributed to high heritability estimates (Table 5.1) for grain yield (54%) and ear prolificacy (83%) that resulted in effective phenotypic selection of these traits. Bello et al. (2012), reports that high heritability is associated with high genetic gains and it ensures effective breeding progress during trait improvement. A high yielding inbred line can effectively be used as a female parental inbred line during seed production resulting in increased seed production. However it should be observed that inbred line *per se* performance does not necessarily translate to high *inter se* performance in hybrid combination. Therefore there is need to substantiate productivity of the line with combining ability information. This is discussed in the next Chapter.

Plant aspects such as stalk lodging, plant and ear heights had desired gains observed relative to the inbred checks. This gain can be credited to high heritability (Table 5.1) observed for the traits resulting in effective phenotypic selection of the traits thus ensuring breeding progress. Uhr and Goodman (1995) report excessive rank growth and increased lodging in tropical germplasm under temperate environments as characteristic of lack of adaptability. Therefore gains attained in the current study are highly desirable; the selected introgressed lines had good standing ability that may allow machine harvesting during seed production thereby increasing efficiency. Standing ability is crucial because maize harvesting is mechanised in South Africa. In addition these introgressed lines may also be advanced for breeding as a source of germplasm for good standing ability. Despite the gains attained there

is need for improvement of inferior stalk lodging observed which can be attributed to low genetic variance (Table 5.1) observed for the traits. Improvement of this trait can be realised through additional cycles of introgression and identification of additional temperate donor inbred lines that have better standing ability.

Early physiological maturity attributes such as; anthesis and silking days and grain moisture content attained low gains for the selected introgressed lines. This indicates that the selected introgressed lines are still to achieve the desired early physiological maturity traits that are important for temperate environments. Low gains can be attributed to low genetic variance observed for the introgressed lines (Table 5.1). Gapare et al. (2000) reports that for genetic gain to be achieved there must be enough genetic variation present within the population. In future there is need to increase genetic variation through increasing the number of donor inbred sources if effective gains are to be realised in improving these traits. In addition, the recipient tropical inbred lines used during introgression were also obtained from an established breeding programme; and breeders are known to recycle germplasm during trait improvement which might have contributed to low genetic variation. Early physiological maturity is highly desirable in seed production as it allows early harvesting of seed crop preventing seed quality loss associated with frost damage.

5.5.2 Performance of individual lines using yield as selection criteria

Improvement of desired economic traits in inbred lines is important in increasing efficiency and productivity of the inbred lines in hybrid seed production. Grain yield was used as the main selection trait and selected introgressed lines highlighted that the top ten selections had improved grain yield potential relative to the mean of temperate inbred lines. Most importantly, the gains attained for grain yield were also reflected in the improved yield potential of introgressed lines in both tropical and temperate environments. High grain yield potential in diverse environments is highly preferred in seed production; a cost efficient environment can be selected for utilization during seed production (see chapter 8). Lack of ear prolificacy at individual sites may be attributed to environmental effect. Regrettably this is an important trait

which is associated with increase in yield (Brathwaite and Brathwaite, 2002; Kesomkeaw et al., 2009). In addition, Varga et al. (2004) and Svecnjak et al. (2006) have reported greater efficiency in resource utilization in prolific maize hybrids, suggesting that the trait can be targeted in breeding maize for adaptability. Hence there is need to improve ear prolificacy in the introgressed lines.

Early physiological maturity attributes such as anthesis and silking days, and grain moisture content at harvest had desired gains observed for the selected lines. Introgressed lines 7 (KRC_7), 32 (RARS_7) and 50 (RARS_29) combined high yield potential with early physiological maturity attributes relative to mean of temperate inbred lines. These attributes are important in seed production as early harvesting can be carried out thus ensuring that fields are available in time for subsequent cropping season. In addition this also reduces frost damage on seed that is associated with late harvesting of the crop in temperate environments.

Negligible improvement for plant aspects such as plant and ear heights, root and stalk lodgings relative to mean of temperate inbred lines is a major concern. This indicates that there is need for further improvement of these traits through inclusion of additional temperate donor lines that have better standing ability in the next cycles of introgression. Strong root and strength is highly desirable in temperate environments which are prone to seasonal wind storms. In addition, it also allows use of high population density during production and machine harvesting thus increasing efficiency of seed production. Inbred lines selected using ear prolificacy as the primary selection criteria showed gains similar in trend with the inbred lines selected using grain yield as the primary selection criteria.

5.5.3 Predicted genetic gains

Predicted genetic gains observed for gran yield and ear prolificacy indicates that there was high realised gain (actual genetic gain) achieved through introgression relative to the expected gains (predicted). This indicates that phenotypic selection method was effective in improving grain yield and ear prolificacy. Predicted gains estimated for

improved plant standing ability attributes were in the undesirable direction, an indication of the difficulty in improving these traits. Anthesis and silking days and grain moisture content at harvest had predicted gains that were comparable to realised gains. This was an indication that phenotypic selection was effective in improving these attributes in new progeny lines.

Estimates of genetic variation that ranged from negligible to low indicate the possibility for low genetic gain during selection for the economic traits. This illustrates slow progress that is likely to be experienced in improvement of the new introgressed lines for the desired traits required for warm temperate environments of South Africa. Contrary to this study, Souza et al. (2009), reports that high genetic variation in a population emphasise the possibility of gain during selection, thus provide an opportunity for improvement of traits. Low coefficient of genetic variation observed in the economic traits indicates the difficulty of achieving selection gain in the introgressed lines. Al-Tabbal (2012) reports that coefficient of genotypic variation reveal the extent of genetic variability present in the genotypes. Therefore low values observed indicated the possibility of low genetic gain realised during selection. High coefficient of genotypic variation accompanied by high estimates observed would be preferred as it would provide reliable estimates of genetic gain expected through selection.

5.6   Conclusion

Positive genetic gains were realised for grain yield (5%) and ear prolificacy (46%) relative to the population mean and the mean of checks. Plant aspects such as plant and ear height, root and stalk lodging had gains ranging from 2% to 11%. Regrettably, plant attributes contributing towards early physiological maturity; anthesis and silking days and grain moisture content at harvest had low gains achieved. This illustrates that there is need to further improve these traits to enhance adaptability and use of the inbred lines in seed production. There were outstanding introgressed inbred lines such as inbred line 71-DMLF7_88 that combined early physiological maturity, high ear prolificacy and grain yield potential at par with the

mean of temperate checks. General conclusion was that introgression of temperate germplasm into tropical elite inbred lines was effective in attaining genetic gains in improving desired traits that are important for South African warm temperate environments.

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6 Assessment of genetic gains for introgression of temperate genes into tropical elite maize lines: II. Performance *inter se*

Abstract

Elite tropical maize germplasm can be improved for adaptation to temperate environments. The objective of this study was to determine the genetic gains for grain yield and its components achieved by the introgression of temperate germplasm in tropical elite inbred lines. The resultant introgressed lines were crossed to four tropical elite inbred line testers to obtain single cross hybrids. The single cross hybrids were evaluated in an alpha lattice augmented design at three sites in South African temperate environments. Grain yield and ear prolificacy had positive realised genetic gains of up to 58% and 26%, respectively relative to population mean and commercial check hybrids. Secondary traits such as anthesis and silking days had gains ranging from 1% to 37%. Negligible gains were attained for stalk and root lodging, and grain moisture content at harvest. Despite the need for further improvement, introgressed lines performance *inter se* indicated significant improvements of grain yield potential following one breeding cycle. Exceptional hybrids like 12C20264, 12C22766, 13XH349 and 11C1774 combined high yield potential with low grain moisture at harvest and improved standing ability relative to commercial check hybrids. Parents of these hybrids will be advanced in the programme and will be the basis of future breeding for adaptation to temperate environments. In conclusion, introgression strategy was effective for improving tropical elite inbred lines for the desired economic traits.

Keywords: maize hybrids, genetic gain, grain yield, grain yield components, performance *inter se*.

6.1   Introduction

Development of widely productive hybrid varieties which are adaptable to changing environment is paramount for maize breeding programmes. To ensure efficiency and long term sustainability of the programme it should have the ability to produce hybrid varieties that have the desired economic traits for the target environments. According to Malosetti et al. (2013) the success of any breeding programme depends on its ability to provide farmers with maize genotypes with guaranteed superior agronomic performance across environments. In the current study, focus was on development of tropical germplasm that is adaptable to the South African warm temperate environments relative to adapted commercial check hybrids.

Tropical hybrids that are directly introduced into temperate environments are characterized by lack of adaptability. According Abadassi and Herve (2000) and Uhr and Goodman (1995) tropical germplasm is characterized by: excessive rank growth, late flowering, excessive lodging, high grain moisture content at harvest and late maturity, when directly introduced into temperate environments. In this study a common donor temperate inbred line was used to enhance adaptability of tropical inbred lines in South African environments. Selection of the introgressed lines was based on key economic traits that are highly desirable for the South African market namely: good plant standing ability reduced rank growth, early flowering, and low grain moisture content at harvest and high yield. In maize breeding programmes identification of F_1 hybrids with superior agronomic performance is fundamental importance towards increasing productivity in target environments (Schrag, 2008). Therefore, in this current study it was sensible to establish introgressed lines *inter se* performance in single cross hybrids generated from testcrossing introgressed lines to tropical elite inbred line testers. The test crosses were evaluated relative to the adapted commercial check hybrids in South African warm temperate environments. Performance *per se* of the introgressed lines, which is also important for predicting seed production potential, is discussed in Chapter 5.

A number of studies have reported the importance of enhancing genetic gain in hybrid maize breeding programmes with particular emphasis on grain yield increase. Bello et

al. (2012) using ten open pollinated maize varieties found high genetic gain for grain yield, number of ears per plant, plant and ear heights. Significant genetic gains have been reported in a temperate maize population by Hallauer and Carena, (2012). Phillip (2007) also reported genetic gain for grain yield and its components in temperate maize germplasm through incorporating tropical genes. Windhausen et al. (2012) using 20 maize hybrids reports predicted increase in genetic gain for grain yield and anthesis date. While, Vashistha et al. (2013) reports moderate estimates of genetic gain for grain yield, plant and ear height using 20 maize hybrids. In this current study assessment of genetic gains for introgression of temperate germplasm into tropical elite maize inbred lines with focus on desirable economical traits for the South African temperate environments was carried out.

The objectives of the current study were to assess: genetic gains for grain yield and its components, as a basis of gaining information for response to selection; and to establish introgressed lines *inter se* performance in single cross hybrids. The results would be used to devise breeding strategy for introgressing temperate germplasm into tropical elite inbred lines to improve adaptation in warm temperate environments, such as South Africa.

6.2   Material and Methods

6.2.1 Germplasm

The experimental material comprised of single cross maize hybrids generated from test crossing Introgressed lines to four tropical elite inbred line testers T1, T2, T3 and T4, representing maize germplasm from two tropical heterotic groupings P and N were used. Due to the large number of the single cross hybrids involved and for convenience of the study, the single cross hybrids were divided into two populations that were designated Population A and Population B. Population A comprised 280 experimental single cross hybrids including four commercial hybrid checks; temperate (PAN6611, PAN6Q445B; tropical hybrids PAN67 and SC633). Population B consisted

of 160 experimental single cross hybrids including three commercial hybrid checks (PAN6611, PAN6Q445B and SC633).

6.2.2 Experimental design

The single cross hybrids were evaluated relative to the adapted commercial hybrid checks. Population A was replicated over two sites namely Ukulinga and Cedara Research Stations. Detailed description of the experimental sites is depicted in Table 2.4 (see Chapter 2). Population B was replicated over three sites namely: Ukulinga, Cedara and Potchefstroom Research Station in South Africa (Table 2.4).

The experimental design was an augmented alpha lattice design (Lin and Poushinsky, 1983; Scott and Miliken, 1993; Spehar, 1994). In Population A, 282 entries (experimental and check) were randomly assigned into 20 blocks; in each block 14 entries were included with two repeating checks (PAN3Q740 and PAN67). Commercial check entries SC633, PAN6227 and PAN6Q445B were also included as non-repeated checks in the trial. In Population B, 162 entries (experimental and check) were randomly assigned into 16 blocks; in each block 10 entries were included with two repeating checks (PAN6611 and PAN6Q445B); and commercial entry (SC633) was used as non-repeated checks.

6.2.3 Field layout and agronomic management

At Ukulinga Research Station each entry was planted to single row plots of 5m length, spaced at 0.3m in-row and 0.75m between row spacing to achieve a total plant population density of at least 44 000 plants ha^{-1}. At Cedara Research Station single 5m row-plots, in-row spacing 0.3 and row spacing of 0.9m were used to achieve a plant stand of at least 37 000 plants ha^{-1}. While at Potchefstroom Research Station single row plots of 6.6m length, spaced at 0.25m in-row and 1.5m between row spacing were employed to attain a total plant population density of at least 26 000 plants ha^{-1}. Standard cultural management practices for growing maize were carried out at all the sites. Irrigation was only applied to achieve uniform establishment and

also to supplement rainfall as and when necessary. Fertilizer application was done at a rate of: 120kg Nitrogen (N), 33kg Phosphorous (P), and 44kg Potassium (K) at Cedara, Ukulinga and Potchefstroom Research Stations.

6.2.4 Variables measured

Comprehensive data was collected at all the sites using standard procedures used at CIMMYT (1985) for the following traits: anthesis and silking days, plant and ear heights, percentage stalk and root lodging, number of ears per plant, percentage grain moisture content at harvest and grain yield. The traits were measured as described in Chapter 2.

6.3   Statistical analyses

6.3.1 Analysis of variance

Data for grain yield and other agronomic traits from individual sites and combined sites was subjected to general analysis of variance (ANOVA) using PROC GLM of SAS (SAS Institute Inc., 2010). Before a combined analysis of variance was carried out, test for homogeneity of variance following Levene test and Welch's test was conducted using GLM procedure of SAS (SAS Institute Inc., 2010).

The linear statistical model for the combined data was as follows:

$$Y_{ijk} = \mu + B_i + C_j + X_k(C) + Eijk$$

Where**:** *Y_{ijk} = observed inbred response; μ = overall trial mean; B_i = effect of the ith block; I =1 …6; C_j = effect of the jth hybrid control; j = 1.2; X_k (C) = effect of the experimental hybrid within checks; k = 1… 160 (Population A) and 280 (Population B); E_{ijk} = random experimental error. The block effects were treated as random while the hybrid main effects were considered fixed.*

6.3.2 Estimation of genetic parameters

The data measurements were used to compute and estimate genetic parameters at 10 % selection intensity for grain yield and its components in Population A and B as described in Chapter 5.

6.4 Results

6.4.1 Analysis of variance

Mean squares of grain yield and its components for maize hybrid Population A and B are presented in Table 6.1. Experimental entries were observed to be significantly different (P<0.01) for all the economic traits excluding ear height and root lodging in Population A. In Population B experimental entries were observed to be significantly different (P<0.01) for all the economic traits. Check entries were significant (P<0.01) for all the traits apart from root lodging, while in Population B all the economic traits were significant except grain moisture content at harvest. Environment effect was observed to be significantly different (P<0.01) for all the economic traits except plant height and stalk lodging in Population A for the check hybrids. Experimental entries had significant environment effect observed for; anthesis and silking days root and stalk lodging, ear prolificacy and grain yield in Population B. Mean of squares of grain yield and its components for Population B showed significant (P<0.05) environment effect on all the economic traits excluding ear height for the check entries. Significant (P<0.05) environmental effect for the experimental hybrids was observed for anthesis days and silking days, stalk and root lodging, ear prolificacy, grain moisture content at harvest and grain yield.

Table 6. 1: Combined analyses of mean squares for grain yield and its components for Population A and B

	Maize hybrid Population A over 2 sites					
Trait/Source of variation	Site	Check	X(Check)	Site*Check	Site*X(Check)	MS(Error)
Anthesis days	111.21***	76.00***	12.04***	103.58***	9.91**	2.42
Silking days	133.16***	96.47***	12.21***	127.76***	10.47***	2.81
Plant height (m)	1.19***	0.75***	0.042**	0.043	0.02	0.02
Ear height (m)	0.48***	3.78***	0.029	0.11**	0.02	0.02
Stalk lodging (%)	11170***	1899.34***	219.21***	1539.93***	159.60***	56.73
Root lodging (%)	1791.36***	108.27	58.83	75.18	60.09**	41.61
Ears per plant (No)	0.34***	2.63***	0.09***	0.10***	0.05***	0.02
Moisture content (%)	22.21***	91.99***	1.97***	7.18***	1.17	0.88
Grain yield (t ha^{-1})	154.26***	181.09***	9.64***	40.21***	5.44**	2.98

	Maize hybrid Population B over 3 sites					
Anthesis days	2829.25***	38.25***	13.08***	68.84***	9.43***	4.06
Silking days	2364.02***	15.26*	11.734***	47.14***	8.58***	3.97
Plant height (m)	12.34***	0.21**	0.056***	0.091*	0.03	0.03
Ear height (m)	0.53***	0.17***	0.040***	0.026	0.017	0.01
Stalk lodging (%)	2627.10***	1083.76***	223.29***	338.60*	193.41***	66.37
Root lodging (%)	295.11***	113.40***	39.26***	60.17***	38.32***	3.74
Ears per plant (No.)	3.69***	4.87***	0.13***	0.80***	0.080*	0.06
Grain moisture	927.94***	1.32	5.66***	31.13***	5.49***	1.6
Grain yield (t ha^{-1})	1375.45***	167.32***	1.87**	13.11**	1.27*	1.73

*, **, *** indicates the data is significant at P≤ 0.05, P≤0.01, P≤ 0.001; grain moisture, percentage grain moisture content at harvest; X (Check), experimental hybrids single cross hybrids nested within checks

6.4.2 Realised genetic gain for Population A

Genetic gains realised due to introgression of temperate germplasm in elite tropical lines is interpreted relative to the mean of population (realised genetic gain 1), mean of best commercial check hybrid (realised genetic gain 2) and mean of commercial check hybrids (realised genetic gain 3). Generally the estimates of grain yield

potential and its components of the selected 10% of the hybrids was superior to the population mean and mean of the commercial check hybrids across sites (Table 6.2). The main primary trait, grain yield, actually illustrates that the selected hybrids were superior to the better check hybrid at Cedara Research Station (Table 6.3), but they were inferior by about 9% at Ukulinga Research Station (Table 6.3). A similar trend was also observed for ear prolificacy.

There were also significant gains across sites for secondary traits such as: anthesis and silking days, root and stalk lodging which were reduced by 1% to 37%, respectively, with respect to population mean (Table 6.2). However, there was only a marginal improvement over the mean of commercial check hybrids for these traits, except stalk lodging (5%). There was no improvement of plant attributes, such as plant and ear height which were larger than the population mean (Table 6.2). In contrast, general significant gains ranging from 2% to 21% were observed at Cedara and Ukulinga Research Station stalk lodging, plant and ear heights, anthesis and silking days which were larger than population mean and mean of commercial check hybrids (Table 6.3). The grain moisture content at harvest of selected hybrids was generally above the mean of population and commercial check hybrids

6.4.3 Mean performance of individual hybrids

Within the top selections there were six hybrids that outperformed the commercial hybrids (Table 6.4). The top four (43-12C20264, 80-12C20628, 225-12C22785 and 246-11C1774) hybrids displayed significantly higher yield potential than the commercial check hybrids (P≤0.05). All top hybrids were significantly (P≤0.05) better yielding than the mean of checks (Table 6.4). Another important trait for temperate hybrids is high ear prolificacy. In this regard there was significant improvement because in the top ten there were four hybrids with better ear prolificacy relative to the standard hybrids (P≤0.05).

The other attribute of temperate hybrids is early physiological maturity which is reflected by days to pollen shedding and silk emergence (flowering days), and low

grain moisture content at harvest. Therefore there is concern that the selected hybrids performed poorly with respect to grain moisture content at harvest (Table 6.4) as they exhibited higher grain moisture content at harvest than the population mean. However hybrid 43-12C20264 was outstanding because it combined high yield potential with low grain moisture content at harvest across sites. It displayed a similar trend for the flowering days. Regrettably a general similar trend was observed for grain moisture content at harvest and flowering days at Ukulinga Research Station (Table 6.5) relative to the mean of population and the mean of commercial check hybrids. However, hybrid 246-11C774 exhibited exceptional low grain moisture content at harvest combined with high grain yield potential relative to the mean of the population and mean of commercial check hybrids. This hybrid would therefore qualify for advancement in the breeding programme.

Plant traits such as plant and ear heights, stem and root lodging are very important in warm temperate environments. In general plants of short stature are preferred. Although the top most hybrids were taller than standard commercial check hybrids there were a few but low yielding hybrids that performed better than the standard checks across sites (Table 6.4). A comparable trend was also observed at Ukulinga Research Station with the exception of hybrid 89 (12C20628) that combined top grain yield potential with good plant stature (Table 6.6). With respect to standing ability the top hybrids were generally inferior to the commercial hybrids, but there were hybrids (240-11C1483 and 272-11C2234) which performed better than the standard hybrids but did not perform well across sites (Table 6.4). Four hybrids 60-12C20553, 61-12C20558, 92-12C20684 and 144-12C21710 exhibited good stalk strength compared to the mean of population and the mean of commercial check hybrids at Cedara Research Station (Table 6.5). Ten hybrids exhibited good root strength relative to the population mean and the mean of commercial hybrids at Ukulinga Research Station (Table 6.6). In fact the top three hybrids (89-12C20628, 75-12C20595 and 225-12C22785) combined high yield potential with good root strength.

Table 6. 2: Estimates of realised and predicted gain of grain yield and its components of top performing hybrids from Population A at 10% selection intensity across 2 sites

	Combined sites													
	Realised genetic gain							Predicted genetic gain						
Traits	MS	MP	MBC	MCS	RG 1(%)	RG 2(%)	RG 3(%)	δ^2_g	CGV (%)	h^2 (%)	CV	CGV/CV	St Dev	GG	GG (%)
AD	77.87	78.68	77.60	77.16	-1.02	0.35	0.92	2.69	2.11	39.90	1.74	0.01	1.34	0.94	1.21
SD	77.83	79.11	81.31	77.62	-1.62	-4.28	0.27	3.19	2.30	43.99	5.21	0.00	1.39	1.08	1.38
PH	2.84	2.77	2.76	2.79	2.58	2.87	2.01	0.02	4.52	64.33	8.03	0.01	0.09	0.10	3.59
EH	1.39	1.34	1.30	1.26	3.78	6.26	9.84	0.08	20.21	89.15	10.96	0.02	0.08	0.13	9.05
SL	10.68	11.82	1.70	11.25	-9.59	529.22	-5.00	17.13	38.74	16.67	66.87	0.01	5.46	1.60	15.00
RL	2.61	4.13	0.33	2.58	-36.92	686.99	1.06	2.44	59.90	7.95	210.92	0.00	2.07	0.29	11.10
EPP	1.48	1.41	1.62	1.32	5.06	-8.64	11.81	0.06	16.07	86.60	9.01	0.02	0.13	0.20	13.42
MC	16.48	15.98	15.84	15.45	3.10	4.05	6.66	1.97	8.52	87.88	5.90	0.01	0.65	1.01	6.10
GY	13.04	9.53	13.77	11.39	36.85	-5.27	14.54	3.16	13.62	57.19	18.07	0.01	2.56	2.58	19.75

MS -mean of sampled population; MP- mean of total population; MBC- mean of better check; MCS -mean of all checks; RG 1 - percentage realised gain 1; RG 2 - percentage realised gain 2; RG 3 –percentage realised gain 3; δ^2_g –genetic variance; CGV -coefficient of genotypic variation; h^2 (%) – percentage heritability; CV- coefficient of variance; St dev- standard deviation; GG-genetic gain; GG (%) –percentage genetic gain; AD, anthesis days; SD, silking days; PH, plant height (cm); EH, ear height (cm); SL, percentage stalk lodging; RL, percentage root lodging; EPP, number of ears per plant (ear prolificacy); MC, percentage grain moisture content at harvest; GYD, grain yield (t ha^{-1})

Table 6. 3: Estimates of realised gain and predicted gain of grain yield and its components of top performing hybrids from population A at 10% selection intensity at individual sites

	Cedara Research Station														
	Realised genetic gain							Predicted genetic gain							
Traits	MS	MP	MBC	MCS	RG1 %	RG2 %	RG3 %	δ^2_g	CGV (%)	h^2 (%)	CV	CGV/CV	St Dev	GG	GG (%)
AD	77.54	77.18	79.08	77.7	0.46	-1.94	-0.21	0.25	0.65	10.50	1.51	0.00	0.97	0.18	0.23
SD	76.59	76.27	78.08	76.95	0.42	-1.9	-0.46	0.15	0.51	12.78	1.47	0.00	0.71	0.16	0.21
PH	2.52	2.72	2.69	2.69	-7.33	-6.61	-6.31	0.02	6.23	92.86	5.86	0.01	0.13	0.21	7.82
EH	1.25	1.27	1.25	1.17	-1.32	0.4	7.51	0.07	21.79	60.68	12.38	0.02	0.10	0.10	8.10
SL	17.52	17.72	5	22.25	-1.16	250.36	-21.27	184.90	77.62	63.13	54.64	0.01	9.17	10.19	57.49
RL	0.91	0.29	0.28	0.89	219.29	231.82	2.82	0.03	19.66	1.45	120.22	0.00	0.36	0.01	3.25
EPP	1.46	1.34	1.75	1.33	8.74	-16.4	9.43	0.05	15.19	74.05	9.01	0.02	0.15	0.20	14.55
MC	16.84	15.53	15.41	15.04	8.49	9.31	12.03	3.75	11.50	77.52	5.34	0.02	0.83	1.14	7.33
GY	12.87	9.27	12.84	10.37	38.79	0.21	24.13	8.14	22.17	71.06	14.79	0.02	1.17	1.46	15.78
	Ukulinga Research Station														
Traits	MS	MP	MBC	MC	RG1 %	RG2 %	RG3 %	GV	CGV (%)	h2 (%)	CV	CGV/CV	St Dev	GG	GG (%)
AD	78.48	80.17	75.63	76.63	-2.11	3.77	2.42	8.58	3.73	44.96	1.92	0.02	2.36	1.87	2.33
SD	78.48	80.17	75.63	76.63	-2.11	3.77	2.42	10.74	4.18	50.27	2.96	0.01	2.36	2.09	2.6
PH	2.91	2.83	2.95	2.93	2.79	-1.4	-0.81	0.02	5	56.38	5.2	0.01	0.12	0.12	4.21
EH	1.45	1.4	1.48	1.39	3.75	-1.57	4.82	0.13	25.23	100	10.53	0.02	0.11	0.19	13.82
SL	5.06	5.21	0.13	1.5	-2.82	394.15	237.35	3.34	36.12	4.51	124.52	0	4.01	0.32	6.12
RL	6.77	5.98	4.15	6.18	13.21	63.1	9.61	6.72	38.29	6.16	151.67	0	4.23	0.46	7.67
EPP	1.56	1.47	1.63	1.34	6.63	-3.88	17.06	0.09	19.34	100	9.2	0.02	0.16	0.28	19.19
MC	16.57	16.38	15.54	15.69	1.21	6.67	5.65	1.67	7.79	100	4.43	0.02	0.87	1.53	9.35
GY	15.55	9.82	17.06	13.04	58.4	-8.83	19.27	3.86	12.63	36.41	17.38	0.01	2.07	1.33	13.51

MS -mean of sampled population; MP- mean of total population; MBC- mean of better check; MCS -mean of all checks; RG 1 - percentage realised gain 1; RG 2 - percentage realised gain 2; RG 3 –percentage realised gain 3; δ^2_g –genetic variance; CGV -coefficient of genotypic variation; h^2 (%) – percentage heritability; CV- coefficient of variance; St dev- standard deviation; GG-genetic gain; GG (%) –percentage genetic gain. AD, anthesis days; SD, silking days; PH, plant height (cm); EH, ear height (cm); SL, percentage stalk lodging; RL, percentage root lodging; EPP, number of ears per plant (ear prolificacy); MC, percentage moisture content at harvest; GYD, grain yield (t ha^{-1})

6.4.4 Predicted genetic gain for Population A

Results indicate a predicted gain of 19.75% and 13.42% for grain yield and ear prolificacy, respectively, for selected hybrids across sites (Table 6.2). The levels of predicted gains were similar at Cedara and Ukulinga Research Stations (Table 6.3). The gains in secondary traits such as anthesis and silking days, plant and ear heights, stalk and root lodgings were not in the desired direction (Table 6.2 and Table 6.3). Grain moisture content at harvest of selected hybrids was generally above the mean by 6% to 9%.

Negligible genetic variation was observed for ear prolificacy, plant and ear height across sites (Table 6.2). The remaining traits had low genetic variation ranging from 1.97 to 17.44 for grain moisture content at harvest and stalk lodging, respectively. Coefficient of genotypic variation and heritability estimates ranged from low to high for the economic traits (Table 6.2). The majority of the traits had low coefficient of variation except stalk and root lodging that had high coefficient of variation estimates, 66.78% and 210.92%, respectively. A comparable trend was also observed for the economic traits at individual site (Table 6.3).

Table 6. 4: Summary of grain yield and its components of top performing hybrids from Population A at 10% selection intensity across sites

Entry	GYD	EPP	MC	AD	SD	PH	EH	SL	RL
225	14.89	1.28	17.07	80.35	80.03	2.95	1.44	18.26	2.88
89	14.78	1.64	17.76	78.73	77.93	#	1.50	6.92	4.21
246	14.67	1.78	15.84	76.35	#	2.84	1.40	13.11	0.40
43	14.03	1.35	15.00	76.60	76.46	2.93	1.32	4.69	9.24
75	13.81	1.68	16.31	77.85	77.18	2.73	1.47	6.06	3.99
260	13.78	1.45	16.20	78.85	78.83	2.86	1.36	18.44	0.44
277	13.71	1.19	16.70	77.98	78.31	2.99	1.53	1.35	2.54
45	13.44	1.82	16.30	78.60	78.56	2.90	1.34	7.01	0.22
146	13.30	1.54	17.01	76.98	77.13	2.91	1.49	7.27	1.88
61	12.92	1.67	15.72	80.35	79.68	2.79	1.40	2.93	1.84
41	12.92	1.30	17.75	76.73	#	2.98	1.48	5.37	1.75
254	12.89	1.33	15.65	77.35	76.79	#	1.53	15.77	1.15
137	12.77	1.56	18.01	79.85	79.13	#	1.56	2.27	6.91
271	12.72	1.50	17.06	77.73	77.03	#	1.25	21.60	1.25
253	12.70	1.81	16.95	78.23	80.56	2.85	1.31	13.90	5.50
40	12.69	1.13	16.17	78.48	77.46	#	1.35	34.89	0.70
138	12.62	1.40	18.41	81.35	81.93	2.96	1.57	3.96	9.08
14	12.62	1.33	15.71	77.35	77.33	2.84	1.28	28.69	0.37
245	12.56	1.33	16.26	76.60	77.06	2.85	1.27	10.54	2.54
256	12.53	1.40	15.80	76.10	#	2.74	1.35	13.83	0.37
92	12.45	1.70	15.27	77.35	76.10	2.73	1.39	4.18	1.40
272	12.43	2.05	16.40	76.60	76.36	2.61	1.38	1.37	1.07
263	12.43	1.35	16.55	75.98	76.81	2.78	1.17	1.57	2.54
240	12.40	1.51	16.41	76.73	76.03	2.73	1.13	0.90	1.25
278	12.38	1.29	14.81	76.35	76.56	2.87	1.42	24.93	4.91
20	12.36	1.00	16.52	78.10	76.92	2.86	1.17	14.32	2.26
29	12.28	1.64	16.62	78.23	77.67	2.79	1.32	7.66	0.45
139	12.17	1.33	17.11	78.73	77.88	2.84	1.62	7.34	1.88
Mean of selected	13.04	1.48	16.48	77.87	77.83	2.84	1.39	10.68	2.61
Mean population	9.53	1.41	15.98	78.68	79.11	2.77	1.34	11.82	4.13
Check 1 (PAN3Q740 temperate)	7.58	1.03	14.11	77.95	76.70	2.60	0.90	2.63	0.99
Check 2 (PAN67-tropical)	11.82	1.35	17.04	76.75	75.93	2.90	1.42	15.73	4.09
Check 3 (SC633 tropical)	12.38	1.29	14.81	76.35	76.56	2.87	1.42	24.93	4.91
Check 4 (PAN6Q445B temperate)	13.77	1.62	15.84	77.60	81.31	2.76	1.30	1.70	0.33
Mean of checks	11.39	1.32	15.45	77.16	77.62	2.79	1.26	11.25	2.58
LSD$_{(0.05)}$	0.12	0.01	0.07	0.11	0.12	0.01	0.01	0.53	0.46
CV (%)	18.07	9.01	5.90	1.74	5.21	8.03	10.96	66.87	210.92
St dev	2.56	0.13	0.65	1.34	1.39	0.09	0.08	5.46	2.07
SE	18.07	9.01	5.90	1.74	5.21	8.03	10.96	66.87	210.92
Pr>F	**	***	**	***	***	*	NS	***	NS

AD, anthesis days; SD, silking days; PH, plant height (cm); EH, ear height (cm); SL, percentage stalk lodging; RL, percentage root lodging; EPP, number of ears per plant (ear prolificacy); MC, percentage moisture content at harvest; GYD, grain yield (t ha^{-1}); SE, standard error, NS, not significant at P= 0.05; *,**,***significant at 0.05; 0.01; 0.001 level, respectively. #, missing data; temperate, temperate germplasm; tropical, tropical germplasm

Table 6. 5: Summary of grain yield and its components of top performing hybrids from Population A at Cedara Research Station

Entry	GY	EPP	MC	AD	SD	PH	EH	SL	RL
60	14.39	1.30	18.46	78.08	78.08	2.42	0.97	5.50	0.28
257	14.09	1.03	18.36	78.08	76.08	2.27	0.76	10.50	5.28
131	13.79	1.79	15.46	78.08	77.08	2.68	1.18	18.50	0.28
61	13.49	1.54	17.66	79.08	77.08	2.44	1.09	5.50	0.28
144	13.24	1.19	17.21	77.58	77.08	2.58	1.31	1.50	0.28
259	13.24	1.56	17.11	75.58	74.58	2.37	0.97	35.00	2.73
43	13.19	1.53	16.11	75.58	75.58	2.91	1.42	11.00	0.28
225	13.19	1.23	17.91	79.08	78.58	2.78	1.32	36.50	0.28
45	13.14	1.86	16.56	78.08	77.08	2.78	1.07	10.50	6.28
1	12.94	1.37	15.51	77.58	77.08	2.68	1.20	6.50	0.28
92	12.89	1.36	15.96	78.08	76.08	2.43	1.12	5.50	0.28
127	12.84	1.75	16.06	83.58	77.58	#	1.39	22.50	0.28
135	12.79	1.52	16.66	78.08	77.08	2.49	1.09	13.50	0.28
137	12.79	1.56	18.51	77.58	77.58	#	1.50	5.00	0.28
138	12.74	1.17	17.86	78.08	76.58	2.76	1.39	14.00	0.28
78	12.64	1.30	14.66	77.08	76.08	2.71	1.36	37.50	0.28
88	12.59	1.60	16.11	79.08	77.58	#	1.38	0.50	0.28
272	12.59	1.81	16.81	76.58	76.58	2.44	1.35	0.00	0.28
37	12.54	1.42	18.91	75.58	75.08	#	1.29	6.50	0.28
38	12.49	1.59	15.11	75.58	75.58	#	1.64	88.00	0.28
75	12.49	1.37	16.16	78.08	77.08	2.41	1.12	11.50	0.28
260	12.49	1.41	16.26	77.58	77.58	2.83	1.38	32.50	2.23
268	12.49	1.27	18.06	77.08	76.08	0.05	1.43	3.00	0.28
8	12.44	1.54	17.46	74.58	75.58	2.89	1.47	33.50	0.28
39	12.29	1.41	17.76	79.08	77.08	2.63	1.01	34.50	0.28
146	12.19	1.38	16.71	79.08	77.58	2.61	1.17	6.50	0.28
41	12.14	1.29	16.31	75.08	75.08	2.95	1.42	11.00	0.28
Mean of selected	12.87	1.46	16.84	77.54	76.59	2.52	1.25	17.52	0.91
Mean of population	9.27	1.34	15.53	77.18	76.27	2.72	1.27	17.72	0.29
Check 1 (PAN3Q740 temperate)	5.88	1.03	13.58	78.05	76.90	2.58	0.91	2.75	0.00
Check 2 (PAN67 temperate)	11.11	1.34	17.24	77.10	76.25	2.87	1.38	28.25	0.55
Check 3 (SC633 tropical)	11.64	1.23	13.91	76.58	76.58	2.60	1.13	53.00	2.73
Check 4 (PAN6Q455B temperate)	12.84	1.75	15.41	79.08	78.08	2.69	1.25	5.00	0.28
Mean of checks	10.37	1.33	15.04	77.70	76.95	2.69	1.17	22.25	0.89
LSD(0.05)	0.10	0.01	0.06	0.13	0.08	0.01	0.01	0.68	0.09
CV	14.79	9.01	5.34	1.51	1.47	5.86	12.38	54.64	120.22
St dev	1.17	0.15	0.83	0.97	0.71	0.13	0.10	9.17	0.36
St error	0.08	0.01	0.05	0.11	0.07	0.01	0.01	0.58	0.07
Pr>F	*	***	**	NS	NS	NS	NS	***	NS

AD, anthesis days; SD, silking days; PH, plant height (cm); EH, ear height (cm); SL, percentage stalk lodging; RL, percentage root lodging; EPP, number of ears per plant (ear prolificacy); MC, percentage moisture content at harvest; GYD, grain yield (t ha^{-1}), NS, not significant at P= 0.05; *,**,***significant at 0.05; 0.01; 0.001 level, respectively; #, missing data; temperate, temperate germplasm, tropical; tropical germplasm

Table 6. 6: Summary of grain yield and its components of top performing hybrids from Population A at 10% selection intensity at Ukulinga Research Station

Entry	GYD	EPP	MC	AD	SD	PH	EH	SL	RL
				Ukulinga Research station					
89	19.55	1.55	18.44	78.13	78.13	2.69	1.49	2.88	3.65
246	17.27	1.75	14.54	78.63	78.63	2.96	1.45	5.88	0.65
75	16.96	2.05	18.64	77.13	77.13	3.03	1.86	0.63	18.65
225	16.56	1.32	16.24	81.63	81.63	3.01	1.56	0.13	5.35
271	16.34	1.61	16.39	76.63	76.63	2.97	1.21	7.88	2.35
256	16.14	1.63	16.09	77.13	77.13	2.86	1.47	14.88	1.85
240	16.01	1.63	17.29	75.63	75.63	2.79	1.26	2.88	4.85
108	15.88	1.79	16.34	84.63	84.63	2.92	1.64	0.13	4.15
253	15.68	1.67	18.64	78.63	78.63	2.71	1.35	0.13	4.15
245	15.47	1.25	17.19	76.13	76.13	3.00	1.40	2.88	4.85
263	15.44	1.54	17.79	75.63	75.63	2.99	1.33	2.63	4.85
41	15.41	1.37	18.19	78.13	78.13	2.93	1.50	1.13	12.65
134	15.41	1.36	16.74	76.13	76.13	3.09	1.46	8.88	8.65
154	15.38	1.55	15.64	78.13	78.13	3.05	1.56	15.88	8.65
43	15.30	1.13	12.99	76.63	76.63	2.93	1.22	0.13	17.35
181	15.22	1.60	16.69	78.63	78.63	2.49	1.16	13.88	10.85
139	15.17	1.60	17.09	79.63	79.63	3.15	1.76	2.63	4.85
146	15.10	1.74	17.39	76.63	76.63	2.94	1.70	2.88	4.85
114	14.92	1.23	16.09	80.63	80.63	2.61	1.25	7.88	14.85
251	14.89	1.43	16.69	76.63	76.63	2.94	1.41	2.88	4.85
267	14.85	1.63	12.74	82.63	82.63	2.67	1.05	12.88	0.65
123	14.73	1.66	17.19	82.63	82.63	2.72	1.26	2.88	10.85
61	14.58	1.86	14.59	82.63	82.63	3.11	1.72	4.38	4.85
28	14.57	1.66	17.74	76.63	76.63	3.24	1.79	5.88	4.85
45	14.50	1.78	15.29	79.13	79.13	2.87	1.47	4.88	12.65
91	14.34	1.20	18.04	76.63	76.63	3.00	1.50	6.13	1.15
172	14.20	1.66	16.89	77.63	77.63	2.80	1.49	2.63	4.85
Mean of selected hybrids	15.55	1.56	16.57	78.48	78.48	2.91	1.45	5.06	6.77
Mean of population	9.82	1.47	16.38	80.17	80.17	2.83	1.40	5.21	5.98
Check 1 (PAN3Q740 temperate)	9.28	1.04	14.63	77.85	77.85	2.77	0.89	2.50	2.00
Check 2 (PAN67 tropical)	12.54	1.36	16.85	76.40	76.40	3.02	1.46	3.25	7.70
Check 3 (SC633 tropical)	13.28	1.32	15.74	76.63	76.63	2.99	1.72	0.13	10.85
Check 4 (PAN6Q445B temperate)	17.06	1.63	15.54	75.63	75.63	2.95	1.48	0.13	4.15
Mean of checks	13.04	1.34	15.69	76.63	76.63	2.93	1.39	1.50	6.18
LSD(0.05)	0.12	0.01	0.05	0.11	0.17	0.01	0.01	0.43	0.62
CV	17.38	9.20	4.43	1.92	2.96	5.20	10.53	124.52	151.67
St dev	2.07	0.16	0.87	2.36	2.36	0.12	0.11	4.01	4.23
St error	0.10	0.01	0.04	0.09	0.99	0.01	0.01	0.97	0.53
Pr>F	**	**	**	***	***	***	NS	NS	*

AD, anthesis days; SD, silking days; PH, plant height (cm); EH, ear height (cm); SL, percentage stalk lodging; RL, percentage root lodging; EPP, number of ears per plant (ear prolificacy); MC, percentage moisture content at harvest; GYD, grain yield (t ha^{-1}); NS, not significant at P= 0.05; *,**,***significant at 0.05; 0.01; 0.001 level, respectively; temperate, temperate germplasm; tropical, tropical germplasm

6.4.5   Realised genetic gain for Population B

Overall analysis showed positive gains (25%) for grain yield of the selected hybrids over the population mean; but there was negative gain realised relative to the commercial hybrids (Table 6.7). A comparable trend was observed for ear prolificacy. There was a smaller gain for grain yield and number of ears per plant at Potchefstroom Research Station than at Cedara and Ukulinga Research Station (Table 6.8). The trends for secondary traits such as grain moisture content at harvest, anthesis and silking days were similar to observations in Population A. Contrary to Population A, there was general increase in stalk and root lodging, plant and ear height in Population B.

6.4.6   Mean performance of individual hybrids

Compared to Population A, only two hybrids 112-13XH349 and 95-12C22776 were superior to the commercial hybrid checks in Population B for grain yield potential across sites (Table 6.9). Only one hybrid (72-12C21728) revealed better ear prolificacy than the commercial check hybrids. In general there were at least four hybrids that were better than the commercial check hybrids at Potchefstroom Research Station (Table 6.11) and Ukulinga Research Station (Table 6.10) had one hybrid (153-11C2245) that also combined superior ear prolificacy with high yield potential.   There were no hybrids with better ear prolificacy compared to the commercial check hybrids at Cedara Research Station (Table 6.10).

In Population B, the secondary traits across sites demonstrated that the top hybrids were superior to the commercial hybrid checks for maturity traits such as anthesis and silking days, grain moisture content at harvest and were also superior to their counterparts in Population A (Table 6.4). The best hybrid 112-13XH349 in Population B (Table 6.9) outperformed the standards for standing ability. Population B hybrids (Table 6.9) had low grain moisture content at harvest relative to commercial checks. Similar trend was observed at Cedara Research Station (Table 6.10) and

Potchefstroom Research Station (Table 6.11) with at least five hybrids exhibiting low grain moisture content at harvest relative to standard commercial hybrid checks.

Table 6. 7 Estimates of realised gain and predicted gain of grain yield and its components of top performing hybrids from Population B at 10 % selection intensity across sites

	Combined sites														
	Realised genetic gain							Predicted genetic gain							
Traits	MS	MP	MBC	MCS	RG 1 (%)	RG 2 (%)	RG 3 (%)	$\delta^2{}_g$	CGV (%)	h^2 (%)	CV	CGV/CV	St Dev	GG	GG (%)
AD	80.16	82.27	83.30	82.11	-2.57	-3.77	-2.38	3.71	2.40	43.56	2.45	0.01	1.09	0.84	1.04
SD	79.16	81.54	82.05	80.91	-2.92	-3.52	-2.16	2.25	1.90	36.33	2.44	0.01	1.09	0.70	0.88
PH	2.66	2.55	2.47	2.49	4.24	7.61	6.52	0.00	1.07	6.10	6.63	0.00	0.09	0.01	0.36
EH	1.26	1.25	1.23	1.22	1.08	3.04	3.33	0.00	5.45	42.21	9.64	0.01	0.09	0.07	5.30
SL	13.59	13.48	9.36	9.51	0.83	45.12	42.95	0.06	1.80	76.49	60.45	0.00	5.85	7.88	57.96
RL	2.52	2.31	0.31	0.43	9.25	706.59	484.62	1.79	53.13	55.23	83.83	0.01	1.96	1.91	75.59
EPP	1.27	1.25	1.61	1.30	1.43	-21.26	-2.34	4.53	167.57	30.40	18.79	0.09	0.12	0.06	5.06
MC	16.79	16.71	16.49	16.63	0.50	1.84	1.02	2.56	9.52	82.15	7.57	0.01	0.73	1.06	6.28
GY	6.14	4.89	6.99	6.34	25.43	-12.14	-3.20	0.11	5.46	75.88	26.88	0.00	0.53	0.71	11.53

MS -mean of sampled population; MP- mean of total population; MBC- mean of better check; MCS -mean of all checks; RG 1 - percentage realised gain 1; RG 2 - percentage realised gain 2; RG 3 –percentage realised gain 3; $\delta^2{}_g$ –genetic variance; CGV -coefficient of genotypic variation; h^2 (%) – percentage heritability; CV- coefficient of variance; St dev- standard deviation; GG-genetic gain; GG (%) –percentage genetic gain; AD, anthesis days; SD, silking days; PH, plant height (cm); EH, ear height (cm); SL, percentage stalk lodging; RL, percentage root lodging; EPP, number of ears per plant (ear prolificacy); MC, percentage moisture content at harvest; GYD, grain yield (t ha^{-1})

Table 6. 8: Estimates of realised gain and predicted gain of grain yield and its components of top performing hybrids from Population B at individual sites

			Realised genetic gain								Predicted genetic gain					
Traits	MS	MP	MBC	MCS	RG 1 (%)	RG 2 (%)	RG 3 (%)	δ^2_g	CGV (%)	h^2 (%)	CV	CGV/CV	St dev	GG	GG (%)
Potchefstroom Research Station															
AD	84.63	86.87	91.06	88.00	-2.58	-7.07	-3.84	8.78	3.50	52.81	3.17	0.01	2.04	1.90	2.24
SD	82.91	85.97	88.00	86.38	-3.06	-5.79	-4.02	2.73	1.99	20.06	2.51	0.01	1.82	0.64	0.78
PH	2.32	2.17	1.98	2.11	0.15	17.56	10.13	0.02	5.90	80.99	5.52	0.01	0.09	0.13	5.53
EH	1.20	1.13	1.08	1.13	0.07	10.87	5.32	0.00	3.59	15.92	6.86	0.01	0.07	0.02	1.64
EPP	1.41	1.15	1.36	1.12	0.26	3.40	25.61	0.02	10.40	12.94	24.67	0.00	0.75	0.17	12.13
MC	19.01	20.96	19.11	19.32	-1.96	-0.56	-1.60	3.70	10.12	79.34	10.47	0.01	1.97	2.75	14.47
GY	2.75	1.44	2.56	2.25	1.31	7.33	22.39	0.75	31.53	100.00	34.08	0.01	0.44	0.77	28.15
Cedara Research Station															
AD	77.56	78.07	78.00	78.12	-0.65	-0.56	-0.72	0.10	0.41	8.62	1.16	0.00	0.59	0.09	0.12
SD	76.63	77.19	76.00	76.76	-0.73	0.82	-0.17	0.05	0.28	3.14	2.26	0.00	0.62	0.03	0.04
PH	2.25	2.26	4.52	2.75	-0.51	-50.12	-18.07	0.00	0.13	0.47	55.66	0.00	0.63	0.01	0.23
EH	1.23	1.25	1.24	1.22	-1.70	-1.13	0.49	0.00	2.27	4.76	6.19	0.00	0.09	0.01	0.61
SL	15.79	17.46	16.61	13.78	-9.56	-4.93	14.64	3.62	12.05	65.20	49.25	0.00	7.99	9.17	58.05
RL	3.01	0.71	0.16	0.16	325.76	1732.32	1732.32	0.26	16.95	2.79	150.54	0.00	0.86	0.04	1.40
EPP	1.20	1.28	1.05	1.57	-6.81	14.06	-23.74	0.24	40.74	100.00	12.05	0.03	0.16	0.28	22.98
MC	15.15	15.13	15.49	15.96	0.12	-2.24	-5.10	2.90	11.25	74.70	3.48	0.03	0.82	1.08	7.13
GY	11.65	7.83	12.93	11.32	48.82	-9.88	2.89	5.82	20.71	100.00	13.85	0.02	1.05	1.85	15.86
Ukulinga Research Station															
AD	79.53	80.81	80.31	79.49	-1.58	-0.97	0.05	1.57	1.57	14.86	2.04	0.01	1.82	0.48	0.60
SD	78.94	80.59	80.25	78.83	-2.05	-1.64	0.13	3.83	2.48	42.02	2.51	0.01	1.88	1.39	1.76
PH	2.83	2.73	2.66	2.70	3.51	6.29	4.68	7.24	95.10	72.05	5.92	0.16	0.12	0.15	5.38
EH	1.28	1.26	1.24	1.19	1.59	3.28	7.49	1.03	79.17	37.34	8.66	0.09	0.10	0.07	5.13
SL	9.64	11.58	2.19	4.32	-16.79	340.54	122.92	1.58	13.04	34.29	67.65	0.00	6.55	3.95	41.02
RL	4.46	4.57	0.63	1.03	-2.45	613.13	332.20	0.59	17.24	21.70	58.48	0.00	3.51	1.34	30.08
EPP	1.28	1.15	1.64	1.23	11.09	-21.71	4.36	0.02	12.15	100.00	7.07	0.02	0.13	0.23	17.84
MC	14.12	14.11	14.16	13.77	0.09	-0.24	2.58	0.72	6.01	75.47	5.70	0.01	0.60	0.80	5.68
GYD	6.82	4.43	7.61	6.12	54.04	-10.31	11.49	5.07	32.98	100.00	16.47	0.02	0.78	1.37	20.12

MS -mean of sampled population; MP- mean of total population; MBC- mean of better check; MC -mean of all checks; RG 1 - percentage realised gain 1; RG 2 - percentage realised gain 2; RG 3 –percentage realised gain 3; δ^2_g –genetic variance; CGV -coefficient of genotypic variation; h^2 (%) – percentage heritability; CV-coefficient of variance; St dev- standard deviation; GG-genetic gain; GG (%) –percentage genetic gain; AD, anthesis days; SD, silking days; PH, plant height (cm); EH, ear height (cm); SL, percentage stalk lodging; RL, percentage root lodging; EPP, number of ears per plant (ear prolificacy); MC, percentage moisture content at harvest; GYD, grain yield (t ha^{-1})

6.4.7 Predicted genetic gain for Population B

Predicted genetic gains in Population B (Table 6.7) exhibited lower genetic gains 11.53% and 5.06% for grain yield and number of ears per plant, respectively compared to Population A. In contrast increases in predicted gains ranging from 12% to 28% were observed at all the individual sites (Table 6.8). Similar to Population A, secondary traits; anthesis and silking days, plant and ear heights, stalk and root lodgings had predicted gains which were not in the desired direction (Table 6.7 and Table 6.8). Grain moisture content at harvest also exhibited similar trends to Population A with selected hybrids generally showing grain moisture content at harvest above the mean.

Low genetic variation was observed for traits such as anthesis and silking days, root lodging, ear prolificacy and grain moisture at harvest across sites (Table 6.7). Grain yield had negligible genetic variation; while plant height and ear height had no genetic variation. Coefficient of genotypic variation was observed to be low for the majority of the economic traits excluding root lodging and ear prolificacy, 53.13% and 167.57%, respectively (Table 6.7). Narrow sense heritability estimates ranged from low (6.10%) to high (82.15%) for plant height and grain moisture content at harvest. Root and stalk lodging were the only traits that had high coefficient of variation estimates, 83.83% and 60.45%, respectively. At individual sites, a similar trend was also observed (Table 6.8).

Table 6. 9: Summary of grain yield and its components of top performing hybrids from Population B at 10% selection intensity across 3 sites

Entry	GY	EPP	MC	AD	SD	PH	EH	SL	RL
112	7.60	1.00	16.10	80.80	79.70	2.60	1.10	8.80	0.60
95	7.00	1.40	16.00	80.80	79.70	2.70	1.50	25.80	10.20
123	6.70	1.30	15.80	80.80	79.70	2.80	1.40	10.70	0.80
100	6.40	1.20	15.40	75.30	77.80	2.50	1.30	39.10	0.10
103	6.30	1.20	16.80	81.00	80.20	2.60	1.30	3.40	0.70
128	6.30	1.50	18.00	80.00	79.10	2.70	1.50	13.80	7.40
152	6.10	1.40	15.60	81.10	80.20	2.40	1.10	6.50	0.20
72	5.90	1.60	18.50	81.00	79.30	.	1.20	3.60	0.60
65	5.90	1.30	20.40	80.00	80.10	2.70	1.00	6.00	5.60
110	5.90	1.20	14.60	80.30	79.50	2.70	1.50	24.60	0.20
121	5.80	1.20	17.70	80.60	80.20	2.60	1.20	3.90	0.60
115	5.70	1.30	15.30	78.80	77.70	2.70	1.40	33.70	0.60
76	5.70	1.30	17.80	.	.	2.90	1.30	10.50	0.60
108	5.60	1.30	17.00	79.40	78.10	2.70	1.10	12.80	11.10
145	5.60	1.00	16.70	80.10	79.50	2.40	1.10	11.30	0.60
19	5.60	1.20	16.80	82.40	76.70	2.70	1.20	3.00	0.60
Mean of population	4.90	1.30	16.70	82.30	81.50	2.50	1.20	13.50	2.30
Mean of selected	6.10	1.30	16.80	80.20	79.20	2.70	1.30	13.60	2.50
Check 1 (PAN6611 temperate)	6.10	1.50	16.70	82.10	81.00	2.50	1.20	7.20	0.90
Check 2 (PAN6Q445B temperate)	7.00	1.60	16.50	83.30	82.10	2.50	1.20	9.40	0.30
Check 3 (SC633 tropical)	5.90	0.80	16.70	80.90	79.70	2.60	1.20	12.00	0.10
Mean of checks	6.30	1.30	16.60	82.10	80.90	2.50	1.20	9.50	0.40
St dev	0.50	0.10	0.70	1.10	1.10	0.10	0.10	5.90	2.00
St error	0.20	1.90	0.60	1.30	9.40	0.10	0.10	1.50	0.60
LSD(0.05)	0.00	0.00	0.10	0.20	0.20	0.00	0.00	0.70	0.20
CV	26.90	18.80	7.60	2.50	2.40	6.60	9.60	60.40	83.80
Pr > F	**	**	***	***	***	***	*	***	***

AD, anthesis days; SD, silking days; PH, plant height (cm); EH, ear height (cm); SL, percentage stalk lodging; RL, percentage root lodging; EPP, number of ears per plant (ear prolificacy); MC, percentage moisture content at harvest; GYD, grain yield (t ha^{-1}), temperate, temperate germplasm; tropical, tropical germplasm

Table 6. 10: Summary of grain yield and its components of top performing hybrid from Population B at 10% selection intensity at individual sites

	Ukulinga Research Station										Cedara Research Station								
Entry	GYD	EPP	MC	AD	SD	PH	EH	SL	RL	Entry	GY	EPP	MC	AD	SD	PH	EH	SL	RL
18	7.74	1.02	13.50	79.66	77.50	3.01	1.43	9.16	1.03	112	14.47	0.94	13.84	77.00	76.00	1.70	1.08	6.45	0.16
1	7.42	1.30	11.30	78.66	76.50	2.95	1.07	10.16	1.03	123	12.88	1.36	16.24	77.00	76.00	2.26	1.37	9.73	0.16
153	7.41	1.81	13.95	80.66	79.50	2.86	1.35	18.16	1.03	152	12.61	1.45	16.14	78.00	77.00	3.24	1.07	15.30	0.16
112	7.36	1.08	14.55	80.16	78.50	2.90	1.29	11.16	1.03	100	12.56	1.35	14.54	78.00	76.00	1.70	1.29	39.78	0.16
33	7.28	1.40	15.20	81.66	83.00	2.58	1.32	4.16	1.03	121	12.01	1.06	15.94	77.00	77.00	1.78	1.18	0.83	0.16
16	7.26	1.27	12.40	81.16	80.50	3.06	1.51	8.16	10.03	145	11.79	1.13	14.64	76.00	76.00	1.64	1.08	6.45	0.16
6	7.22	1.00	13.80	81.66	84.00	2.79	1.21	4.16	1.03	95	11.72	1.52	15.34	77.00	76.00	2.11	1.54	51.83	0.16
110	6.94	1.32	13.50	76.16	75.50	3.03	1.47	11.16	1.97	71	11.57	1.13	19.24	77.00	76.00	2.08	1.42	6.45	0.16
55	6.48	1.30	13.00	80.66	81.00	2.36	1.16	4.16	20.03	14	10.90	1.20	12.74	78.00	76.00	1.91	1.09	6.06	0.16
106	6.40	1.44	14.85	83.66	81.50	2.94	1.24	15.16	1.03	38	10.76	1.36	14.44	77.00	77.00	2.11	1.20	33.38	5.72
13	6.38	1.44	15.40	78.16	77.50	2.81	1.25	12.16	4.03	19	10.62	1.04	15.74	77.00	76.00	1.89	1.24	15.09	0.16
53	6.38	0.98	15.40	76.16	75.00	2.59	1.13	9.16	4.97	86	10.51	1.21	14.44	77.00	76.00	0.92	1.17	6.45	33.49
108	6.37	1.57	15.50	76.16	75.00	2.79	1.27	22.16	19.03	106	10.47	0.83	12.64	80.00	79.00	3.04	0.87	7.81	0.16
129	6.21	1.05	15.60	79.66	81.00	2.31	1.05	10.16	1.03	128	10.31	1.22	14.84	78.00	77.00	1.88	1.51	26.94	6.83
159	6.19	1.21	12.70	78.16	77.50	3.49	1.67	3.84	1.97	103	10.30	1.27	16.04	79.00	79.00	3.29	1.27	3.53	0.16
151	6.15	1.37	15.30	80.16	79.50	2.78	1.04	1.16	1.03	72	10.23	1.83	17.34	78.00	77.00	0.89	1.22	14.34	0.16
Mean of population	4.43	1.15	14.11	80.81	80.60	2.73	1.26	11.58	4.57		7.83	1.28	15.13	78.07	77.19	2.26	1.25	17.46	0.71
Mean of sampled hybrids	6.82	1.28	14.12	79.53	78.90	2.83	1.28	9.64	4.46		11.65	1.20	15.15	77.56	76.63	2.25	1.23	15.79	3.01
Check 1 (PAN6611 temperate)	5.95	1.45	14.84	79.00	77.80	2.60	1.12	6.13	1.44		10.25	1.82	16.18	78.58	77.54	1.85	1.20	8.18	0.33
Check 2 (PAN6Q445B temp)	7.61	1.64	14.16	80.31	80.30	2.66	1.24	2.19	0.63		10.79	1.84	16.21	77.79	76.73	1.88	1.23	16.54	0.00
Check 3 (SC633 tropical)	4.81	0.60	12.30	79.16	78.50	2.84	1.22	4.66	1.03		12.93	1.05	15.49	78.00	76.00	4.52	1.24	16.61	0.16
Mean of checks	6.12	1.23	13.77	79.49	78.80	2.70	1.19	4.32	1.03		11.32	1.57	15.96	78.12	76.76	2.75	1.22	13.78	0.16
St dev	0.78	0.13	0.60	1.82	1.88	0.12	0.10	6.55	3.51		1.05	0.16	0.82	0.59	0.62	0.63	0.09	7.99	0.86
St error	0.15	0.01	0.06	0.13	0.16	1.27	0.86	0.55	0.18		0.13	0.02	0.48	0.23	0.16	0.56	6.19	0.59	1.51
LSD(0.05)	0.03	0.01	0.07	0.15	0.18	1.42	0.96	0.15	0.07		0.10	0.01	0.05	0.08	0.15	0.07	0.01	0.88	0.08
CV	16.47	7.07	5.70	2.04	2.51	5.92	8.66	67.65	58.48		13.85	12.05	3.48	1.16	2.26	55.66	6.19	49.25	150.54
Pr > F	NS	NS	NS	**	*	NS	*	**	***		*	*	***	NS	NS	NS	*	*	***

AD, anthesis days; SD, silking days; PH, plant height (cm); EH, ear height (cm); SL, percentage stalk lodging; RL, percentage root lodging; EPP, number of ears per plant (ear prolificacy); MC, percentage moisture content at harvest; GYD, grain yield (t ha^{-1}), temperate, temperate germplasm, tropical, tropical germplasm

Table 6. 11: Summary of grain yield and its components of top performing hybrids from Population B at 10% selection intensity at Potchefstroom Research Station

Entry	GY	EPP	MC	AD	SD	PH	EH
			Potchefstroom Research Station				
117	3.31	1.46	17.47	78.00	76.38	2.38	1.05
89	3.09	1.71	16.62	81.00	81.38	2.48	1.25
95	3.08	1.36	17.54	86.00	85.88	2.28	1.33
113	2.99	1.28	14.19	80.00	78.88	2.43	1.15
61	2.8	1.49	21.07	88.00	86.88	2.28	1.13
29	2.74	1.92	21.25	83.00	83.38	2.43	1.28
11	2.73	1.15	15.43	88.50	87.38	2.43	1.23
51	2.71	1.4	19.87	84.00	84.38	2.43	1.33
20	2.7	1.13	17.72	85.00	85.38	2.33	1.08
119	2.68	1.03	18.81	81.50	80.88	2.28	1.18
111	2.65	1.36	17.65	82.00	79.38	2.18	1.15
19	2.63	1.02	22.45	96.50	80.88	2.38	1.28
50	2.62	1.25	21.6	84.50	82.88	2.18	1.18
76	2.49	1.61	22.39	86.00	85.38	2.38	1.25
83	2.41	1.55	18.89	89.00	86.88	2.08	1.13
128	2.4	1.81	21.12	81.00	80.38	2.28	1.15
Mean of population	1.44	1.15	20.96	86.87	85.97	2.17	1.13
Mean of sampled hybrids	2.75	1.41	19.01	84.63	82.91	2.32	1.2
Check 1 (PAN6611 temperate)	2.24	1.09	19.00	88.94	87.75	2.08	1.08
Check 2 (PAN6Q445B temperate)	2.56	1.36	19.11	91.06	88.00	1.98	1.08
Check 3 (SC633 tropical)	1.94	0.91	19.83	84.00	83.38	2.28	1.25
Mean of checks	2.25	1.12	19.32	88.00	86.38	2.11	1.13
St dev	0.44	0.75	1.97	2.04	1.82	0.09	0.07
St error	0.66	0.35	2.65	3.35	2.63	0.14	0.09
LSD$_{(0.05)}$	0.05	0.03	0.19	0.24	0.19	0.01	0.01
CV	34.08	24.67	10.47	3.17	2.51	5.52	6.86
Pr > F	*	*	*	*	**	ns	ns

AD, anthesis days; SD, silking days; PH, plant height (cm); EH, ear height (cm); SL, percentage stalk lodging; RL, percentage root lodging; EPP, number of ears per plant (ear prolificacy); MC, percentage moisture content at harvest; GYD, grain yield (t ha^{-1});

6.5 Discussion

6.5.1 Analysis of variance of the environments and maize hybrid varieties

The entries (experimental and check) in hybrid maize population A and B had siginificant ($P<0.01$) difference observed for the majority of the traits under study. The interaction between entries and enviornment was also observed to be siginificantly ($P<0.05$) for the majority of the traits. This implies that the entries and genotype-by-environment interaction explained the major contribution to these traits than the environment effect. Environmental effect only accounted for a smaller contribution of phenotypic variation. According to Rasyad et al. 2012 increased influence of genotype-environment interaction may suggest that the genotypes performed differently under diverse environments and their performance was unpredictable across environments. Therefore there is need to carry out multi-locational trials to identify hybrids that have yield stability across target environments.

6.5.2 Realised genetic gain

Introgression of temperate germplasm in tropical elite maize inbred lines was generally effective in attaining realised genetic gains in both primary and secondary traits required in a warm temperate environment. Weng et al. (2008) defines realised genetic gain as actual achievable gain in a breeding programme; and that it is important in establishing effectiveness of the breeding strategy implemented in improving required traits during crop improvement. Grain yield and ear prolificacy are the main primary traits in temperate environments. The selected hybrids had general superior performance relative to the population mean and the mean of the commercial hybrid checks in both populations. General positive genetic gains achieved can be attributed to moderate-high heritability (Table 6.2 and Table 6.7) that ensured effective breeding progress. Similar results of genetic gain attained in breeding programmes have been reported for grain yield by Badu-Apraku et al. (2013) and Vashistha et al. (2013). Despite the commendable gains attained in the current study, there is still need for further introgression of temperate germplasm to

improve these primary traits of selected hybrids as inferior performance was observed relative to the better check (leading hybrid on the market-PAN6Q445B).

Secondary traits such as grain moisture content at harvest and flowering days generally exhibited moderate gains relative to the population mean and the mean of commercial check hybrids. Gains attained can be attributed to high percentage coefficient of genetic variance and heritability in Population A (Table 6.2 and 6.3) and Population B (Table 6.7 and 6.8). This indicates that the traits can effectively be selected for during breeding thus ensuring genetic gain. Bello et al. (2012) reports that breeding programmes prefer high genetic gain that is associated with high heritability estimates to ensure effective progress. Similar results have been reported for secondary traits by: Vashistha et al. (2013) on anthesis and silking interval, plant and ear height; Badu-Apraku et al. (2013) for anthesis and silking days, plant and ear height, ear prolificacy and stalk lodging. Despite the general gains reported in the current study, inferior performance of the selected hybrids relative to the better check and the population mean was observed. This again calls for further introgression of temperate germplasm to attain the desired levels that can exceed the best commercial hybrid.

Plant aspects such as stalk and root lodging generally indicated the need for further introgression due to pronounced poor standing ability observed in the selected hybrids relative to commercial check hybrids. Poor standing ability observed in both populations can be credited to lower genetic variance for root and stalk strength in Population A (Table 6.2 and 6.3) and B (Table 6.7 and 6.8). However poor standing ability can also be attributed to frequent seasonal wind storms experienced in South African temperate environments. El-Badawy (2012), report that breeding progress can be achieved in a population that establishes and maintains sufficient genetic variation. Therefore, in future studies there is need to increase genetic variation of introgressed lines through the use of additional donor inbred lines that have good standing ability. Tropical recipient lines that were used during introgression came from an established breeding programme; and breeders have the tendency to recycle germplasm during crop improvement which results in narrow genetic diversity.

Therefore there is also need to increase genetic diversity of the tropical recipient lines through acquiring tropical germplasm from Consultative Group on International Agricultural Research (CGIAR) institutes such as International Maize and Wheat Improvement Centre (CIMMYT) and International Institute of Tropical Agriculture (IITA). Many temperate inbred lines would then be used as sources of temperate germplasm to introgress desired traits in tropical elite inbred lines.

6.5.3 Performance of individual hybrids

The general trend highlighted that the selected hybrids in both populations out yielded tropical hybrids (SC633 and PAN67) and temperate hybrids (PAN6611 and PAN3Q740) in temperate environments. This illustrates significant genetic gain in yield potential that can be credited to increased adaptability of the hybrids. Most importantly, six selected hybrids; 43-12C20264, 80-12C20628, 225-12C22785 and 246-11C1774 in Population A; and 95-12C22776 and 112-13XH350 outperformed PAN6Q445B; a leading hybrid on the market. This shows that introgression of temperate germplasm was effective for increasing grain yield potential of tropical germplasm in warm temperate environments. Kesomkeaw et al. (2009), reports that there is low genetic diversity for ear prolificacy in tropical germplasm. However genetic gain for ear prolificacy was observed in five selected hybrids in Population A and B. Increase in ear prolificacy of tropical germplasm demonstrates a positive gain in a primary trait that is important for temperate environments.

Important attributes for early physiological maturity and grain moisture content at harvest and flowering days demonstrated that introgression of temperate germplasm in tropical germplasm was generally ineffective in improving these attributes. Selected hybrids had high grain moisture content at harvest and flowered late relative to commercial hybrid checks. According to Abadassi and Herve (2000) lack of adaptability of tropical germplasm in temperate environments is characterized by late flowering and high grain moisture content at harvest. Exceptions were observed in hybrids 43-12C20264, 95-12C22776, 112-13XH349, and 246-11C1774 that combined low grain moisture content, early flowering and high yield potential in

temperate environments. Low yielding is an undesirable attribute. However the desired combination of low grain moisture content and early flowering is a major requirement in temperate environments as it reduces costs that are related to artificial grain drying and losses due to delayed harvesting in particular frost damage. Early harvesting also allows the farmer timely planting of winter crops. Therefore introgressed lines require further advances to improve these traits.

The selected hybrids illustrated that plant aspects such as plant and ear height, stalk and root lodging required further introgression to improve pronounced poor standing ability and increased rank growth relative to commercial hybrid checks. This indicates that the selected hybrids lacked the desired traits that will ensure good standing ability in temperate environments that are also prone to seasonal wind storms. Lewis and Goodman (2003) and Nelson and Goodman (2008), reported that poor plant standing ability and increased rank growth of tropical germplasm in temperate environments indicates lack of adaptability. Nevertheless there were a few low yielding hybrids that exhibited good standing ability.

6.5.4 Predicted genetic gains

Predicted genetic gain for grain yield and number of ears per plant indicated that higher gain was achieved in Population A relative to Population B. However the general trend indicated that predicted genetic gains were higher than the actual (realised genetic gains) achieved. This indicates that the phenotypic selection method used was not effective in achieving the desired breeding progress. Plant aspect; plant and ear heights, root and stalk lodgings illustrate that predicted genetic gain was in the undesirable direction, an indication that attaining actual genetic gain of these traits requires huge breeding effort. A similar trend was also observed for attributes of early physiological maturity, grain moisture content and flowering days. This highlights that breeding tropical germplasm for adaptability in warm temperate environments still has opportunities for further gain.

Negligible to low genetic variation was observed for the majority of the economic for the maize hybrid populations indicates that there is likely to be low genetic gain during selection for the traits, resulting in slow breeding progress in improvement. Traits such as plant and ear height that did not show genetic variation illustrating that phenotypic selection may not achieve any desired genetic gain for the introgressed lines. The majority of the economic traits showed low coefficient of genotypic variation and heritability estimates that ranged from low to high. A low coefficient of genotypic variation indicates difficult of achieving selection gain for these traits. However, Al-Tabbal (2012) reported that coefficient of genotypic variation alone does not provide full scope to assess variation that is heritable. Therefore, coefficient of genotypic variation should be considered along with heritability estimates to provide reliable estimates of the amount of genetic gain to be expected through selection. High coefficient of variation for plant and ear height indicates the need to improve the quality of experiments through minimising error during selection.

6.6   Conclusion

Generally positive desired realised genetic gains were attained for grain yield (58%) and number of ears per plant (26%) relative to population mean and mean of commercial check hybrids. However inferior genetic gains (9%) relative to better commercial hybrid checks were observed. Secondary traits such as anthesis and silking days had desirable realised genetic gains ranging from 1% to 37% relative to the mean of the population while stalk lodging had 5% gain relative to commercial hybrid checks. Grain moisture content at harvest indicated that there was a negligible gain achieved relative to the population mean and mean of commercial checks as selected hybrids had higher grain moisture content at harvest. Stalk and root lodging did not attain the desired realised genetic gain as mean of introgression hybrids was higher than commercial hybrid checks. Despite the need for further improvement, introgressed lines performance *inter se* indicated that significant improvements of grain yield potential and its components following one breeding cycle. Most interesting was the exceptional performance of hybrids such as; 43-12C20264, 95-12C22776, 112-13XH349, and 246-11C1774 that combined high yield potential with low

grain moisture content at harvest and improved standing ability. This indicates that introgression of temperate germplasm in tropical maize elite inbred lines was effective for enhancing adaptability of the tropical elite inbred lines in South Africa. However there is concern that the majority of the selected hybrids performed poorly with respect to plant aspects such as stalk and root lodging and grain moisture content. Therefore there is need to further improve these plants to enhance adaptability of tropical germplasm in temperate environments. Future breeding will emphasize these traits.

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7   Stability assessment of single cross hybrids using GGE-biplot analysis

Abstract

Grain yield potential of new maize hybrid varieties across target environments determines the rate of uptake of these varieties by farmers. Single cross hybrids developed from testcrossing introgressed lines bred from three distinct environments to elite tropical inbred line testers were evaluated. The objective of the study was to assess yield stability and genotype adaptability of the single cross hybrids across the South African warm temperate environments relative to adapted commercial hybrid checks. Field evaluation was carried out using an alpha lattice augmented design. Data of grain yield and its components were subjected to analysis of variance; with grain yield being further subjected to GGE-biplot analysis. GGE-biplots patterns revealed two mega environments in the hybrid maize hybrid trials. Ukulinga Research Station had the ability to clearly discriminate hybrids according to yield potential. Introgressed lines from all selection environments demonstrated good yield potential in hybrid combination with testers T1, T2 and T4. There were six winning genotypes, namely 25(12C19813), 89 (12C20628), 108 (13XH344), 110 (13XH346), 112 (13XH349) and 128 (13XH1060), which can be recommended for future studies in target environments. High yield potential of introgressed lines in hybrid combinations indicated the effectiveness of introgression in improving adaptability in target environments.

Keywords: maize hybrids, GGE-biplots, genotype, genotype-environment interaction

7.1   Introduction

In developing countries, in particular Africa maize (*Zea mays,* L) is a critical and strategic cereal crop grown across regions. Its wide adaptability in target environments has rendered it a staple food crop across tropical, subtropical and temperate regions of the world. In South Africa, a predominantly warm temperate environment, maize is the largest locally produced field crop with increasing food, feed and industrial usage value for the population (Syngenta, 2013). Maize is also regarded as a net earner of foreign currency for the South African economy. Therefore South Africa maize production is a large and lucrative market for breeding programmes operating inside and outside South Africa. This indicates that breeding programmes should ensure release of stable maize hybrid varieties that perform well in the South African warm temperate environments.

In maize breeding the primary objective is to develop hybrids that have high yield potential and adaptability across target environments. According to Balestre et al. (2009), breeders should select high yielding genotypes associated with high yield stability. Mostafavi et al. (2011) reports that targeting of improved cultivar varieties to specific environments is difficult when genotype-by-environment interaction is present, since yield is less predictable and cannot be interpreted based only on genotype and environment means. Genotype-by-environment interaction is defined as the differential ranking of cultivars yields across target environments; resulting in variable performance of cultivars in selected target environments (Crossa et al., 2002). This complicates utilization of maize hybrid varieties across target environments.

In this study, emphasis is on identifying improved tropical introgressed maize inbred lines combinations capable of maximizing maize production potential in South African warm temperate environments and farming systems, thus reduce incidences of crop failure or low yields in unfavourable seasons. Breeding programmes have to develop improved maize varieties for the farmers that have good agronomic performance relative to adapted commercial check varieties in target environments.

Recommendation of improved hybrid varieties in target environments requires these genotypes to be evaluated in several different environments to identify both consistent high yielding and relatively stable genotypes, and areas of specific adaptation (Balestre et al., 2009).

A number of methods have been applied in maize breeding programmes to evaluate adaptability and stability of cultivars in target environments. Two main methods have been consistently used in a number of studies namely: additive main effects and multiplicative interaction (AMMI) analysis (Gauch et al., 2008; Gauch, 2006; Duarte and Vencovsky, 1999); and a modification of the conventional AMMI analysis called genotype (G) and genotype-by-environment interaction (GE) (GGE-biplot) analysis (Yan and Tinker, 2006; Kaya et al., 2006; Yan et al 2000). AMMI and GGE-biplot provide breeders with a tool to efficiently and accurately measure the response of maize hybrid varieties in multiple test environments (Yan et al. 2007). According to Balestre et al. (2009) AMMI analysis interprets the effects of genotypes and environments as additive and GE interaction as multiplicative, by principal component analysis. The GGE-biplot analysis groups the genotype effect which is an additive effect in AMMI analysis together with the GE interaction, multiplicative effect, and analyses these effects by principal components (Kaya et al. 2006).

Genotype, and genotype-by-environment interaction, analysis was carried out in the current study on single cross maize hybrid maize varieties to compare grain yield potential of these genotypes across target environments relative to the adapted commercial check entries. Comparison of grain yield potential of the maize genotypes at different environments or group of environments in South African environments ensured identification and recommendation of genotypes that had higher grain yield potential in each target environment. As a breeder, the main objective is to breed for high grain yield potential, and that the high grain yield potential should be highest or close to the highest, consistently in all locations within the geographical area for which variety will be released (Yan and Tinker, 2006).

The objective of the study was to assess yield stability and genotype adaptability of the single cross hybrids using GGE biplot analysis across the South African warm temperate environments relative to adapted commercial hybrid checks. Productivity data of the hybrids and genetic gains are presented in Chapter 6. In this Chapter, the focus is on stability and adaptation.

7.2   Materials and Methods

7.2.1   Germplasm

The germplasm used in this study is described in Chapter 6.

7.2.2   Experimental design and management

The experiment was designed and managed as described in Chapter 6.

7.2.3   GGE Biplots

Genotype, genotype-environment interaction GGE-biplot analysis was carried out on yield data only. GGE Biplots concept (Yan et al., 2000) was used to visualize the multi-environment trials (MET) data as reported by Kaya et al. (2006). The GGE-biplot showed the first 2 principal components (PC1 and PC2) derived from subjecting environmental centred yield data (yield evaluation due to GGE) to singular value decomposition (Yan et al., 2000). In the current study, genotype-focused scaling was used for visualizing genotypic comparison, with environment-focused scaling for environmental comparison using GGE-biplots (Genstat 14 edition, 2013).

7.3   Results

7.3.1 GGE-biplots

Based on the partitioning of GGE through GGE-biplots, six biplots were plotted for the entries (experimental and checks) Population A GGE-biplots are presented in Figure 7.1 to 7.3, while Population B GGE-biplots are presented in Figure 7.4 to 5.6. The biplot consists of PC1 scores plotted against PC2 scores for both genotypes and environments.

7.3.2 Genotype evaluation based on GGE-biplots for Population A

Visualization of which-won-where pattern of the multi-environment trials data for hybrid maize Population A is depicted through the polygon views of the GGE-biplot (Figure 7.1). The polygon was formed by connecting scores of genotypes that are furthest away from the point of origin resulting in all the other genotypes being confined in the polygon. Eight sectors were formed on this plot with the environments falling into four sectors. Ukulinga Research Station fell into sector 2 demarcated by rays 2-3, with the vertex genotypes for these sectors being experimental single cross hybrid 89 (12C20628). This suggests this was the winner genotype at Ukulinga Research Station. Ukulinga Research Station was the environment that discriminated the genotypes more clearly as shown by higher PC1 scores. The two PC scores accounted for 61.38% and 38.17% of the total GGE variation, respectively. Therefore the biplot explained for 99.55% of the total variation relative to genotype and genotype-environment interaction. Experimental entries 89 (12C20628) was the vertex genotype as it outperformed all the entries at Ukulinga Research Station; while at Cedara Research Station experimental entry 61 (12C20568) was the vertex genotype as reflected by its outstanding yield potential. Check entries 281(PAN3Q740) and experimental entries 132 (12C21233) were among some of the entries that had lower than average yield in Population A. This is indicated by their placement in sector 7 that shows low yielding genotypes.
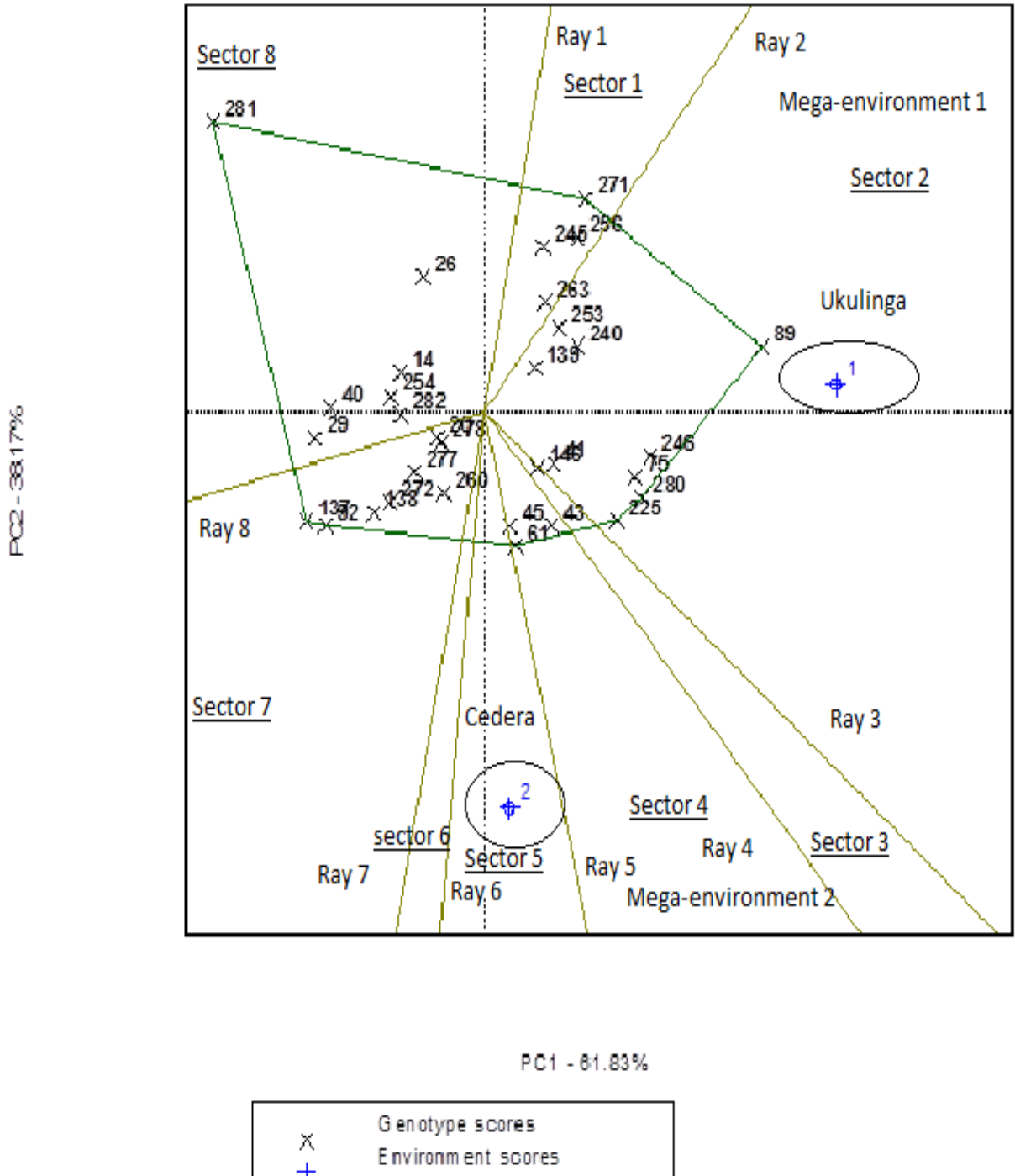
Figure 7. 1: Polygon views of the GGE-biplot based on symmetrical scaling for which-won-where pattern for genotypes and environments for Population A. Details of the environments are given in Table 2.4

In Figure 7.2 the GGE biplot based on genotype-focused scaling for comparison of genotypes with ideal genotypes for Population A indicated that the biplot explained 99.55% of the total variation relative to genotype and genotype-by-environment interaction. The ideal genotype was used for referencing entries for evaluation. Check entry 280 (PAN6Q445B), experimental entries (75-12C20595, 225-12C22785 and 246-11C1774) were shown to be close to ideal genotypes in terms of higher yielding ability and stability relative to the other entries as they almost fell into the centre of concentric circles. Experimental entries 61-12C205528, 45-12C20300, 43-12C20299, 141-12C21609 and 89-12C20628 were the desirable genotypes identified in Population A. As indicated by the placement in the concentric circles for desirable genotypes (Figure 5.2). Check entries 281 (PAN6Q445B) was classified as highly undesirable genotypes in this trial as they were placed furthest away from the ideal concentric circle.
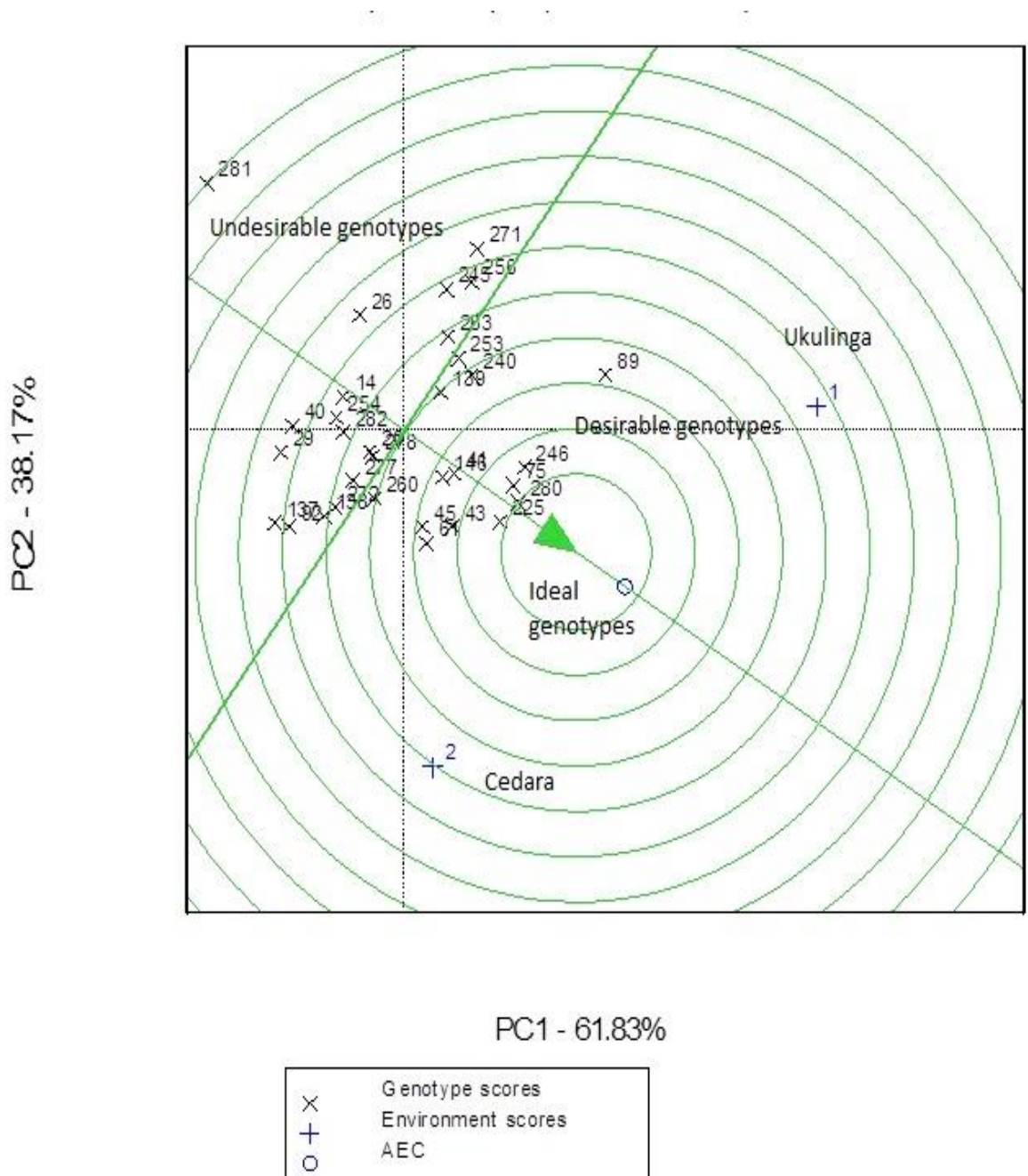
Figure 7. 2: GGE biplot based on genotype-focused scaling for comparison of genotypes with ideal genotype for Population A.

An ideal test environment should be able to discriminate genotypes in a given trial evaluation. Figure 7.3 shows GGE-biplot of PC2 and PC1 based on environment-focused scaling for comparison of the environments with the ideal environment for Population A. The biplot accounted for 99.55% of the total variation relative to genotype and genotype-by-environment interaction. Referencing of ideal environment to Ukulinga and Cedara Research Stations showed that the two environments did not fall in the centre of the concentric circles. Hence, the two environments were not the ideal environment for discriminating yield for entries. However the two environments were placed within the concentric circles that were defined as favourable environments, indicating high yield potential.

Figure 7. 3: GGE-biplot based on environment-focused scaling for comparison of the environments with the ideal environment for Population A. Details of environments are given in Table 2.4.

7.3.3 Genotype evaluation based on GGE-biplots for Population B

A polygon view, visualizing which-won-where pattern of the multi-environmental trials data for Population B is shown in Figure 7.4. The biplot explained 94.48% of the total variation relative to genotype, and genotype-by-environment interaction. Rays of plot divided the polygon into nine sectors, with genotype entries 162 (PAN6Q445B), 110 (13HX346) and 108 (13XH344) defining the two mega environments formed. Experimental entry 162 (PAN6Q445B) was the vertex genotype, out yielding all the genotypes at Ukulinga research station, at Cedara Research Station entry 112 (13HX349) out-yielded the rest of the entries as it was the vertex genotype for mega environment 2. Potchefstroom Research Station had entries 128 (13HX1060) and 161(PAN6611) as best performing hybrids.
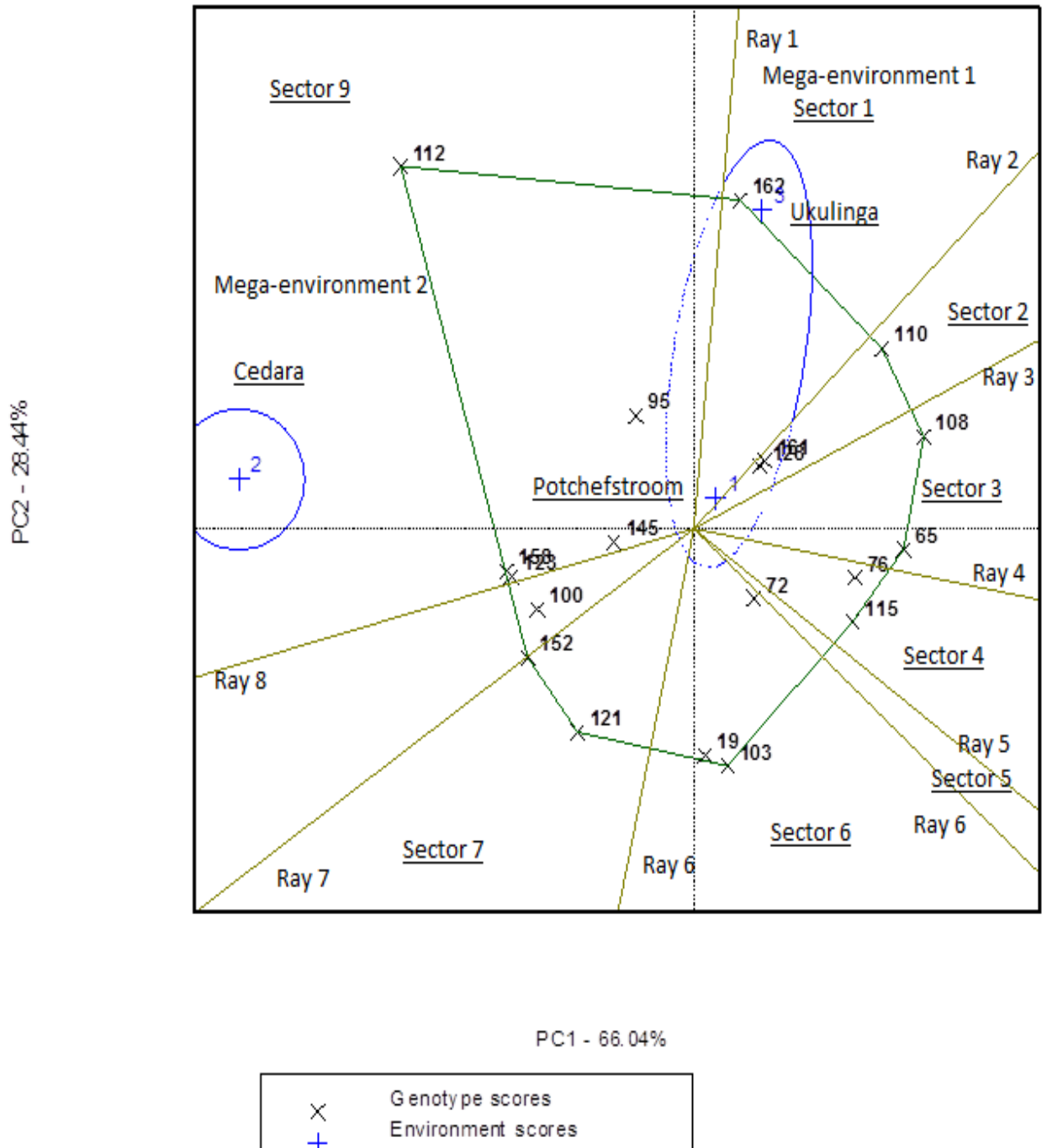
Figure 7. 4: Polygon views of the GGE-biplot based on symmetrical scaling for which-won-where pattern for genotypes and environments for Population B. Details of the environments are given in Table 2.4

Comparison of entries relative to the ideal genotype based on GGE biplot established on genotype-focused scaling for Population B (Figure 7.5). The biplot accounted for 94.48% of the total variation relative to genotype, and genotype-by-environment interaction. Ideal genotype was entry 112 (13XH350) as it almost fell into the centre of concentric circles. Entries 162 (PAN6Q445B) and 95 (12C22776) were the next best desirable genotypes in terms of higher yielding ability and stability. The majority of the entries were classified as undesirable in Population B.
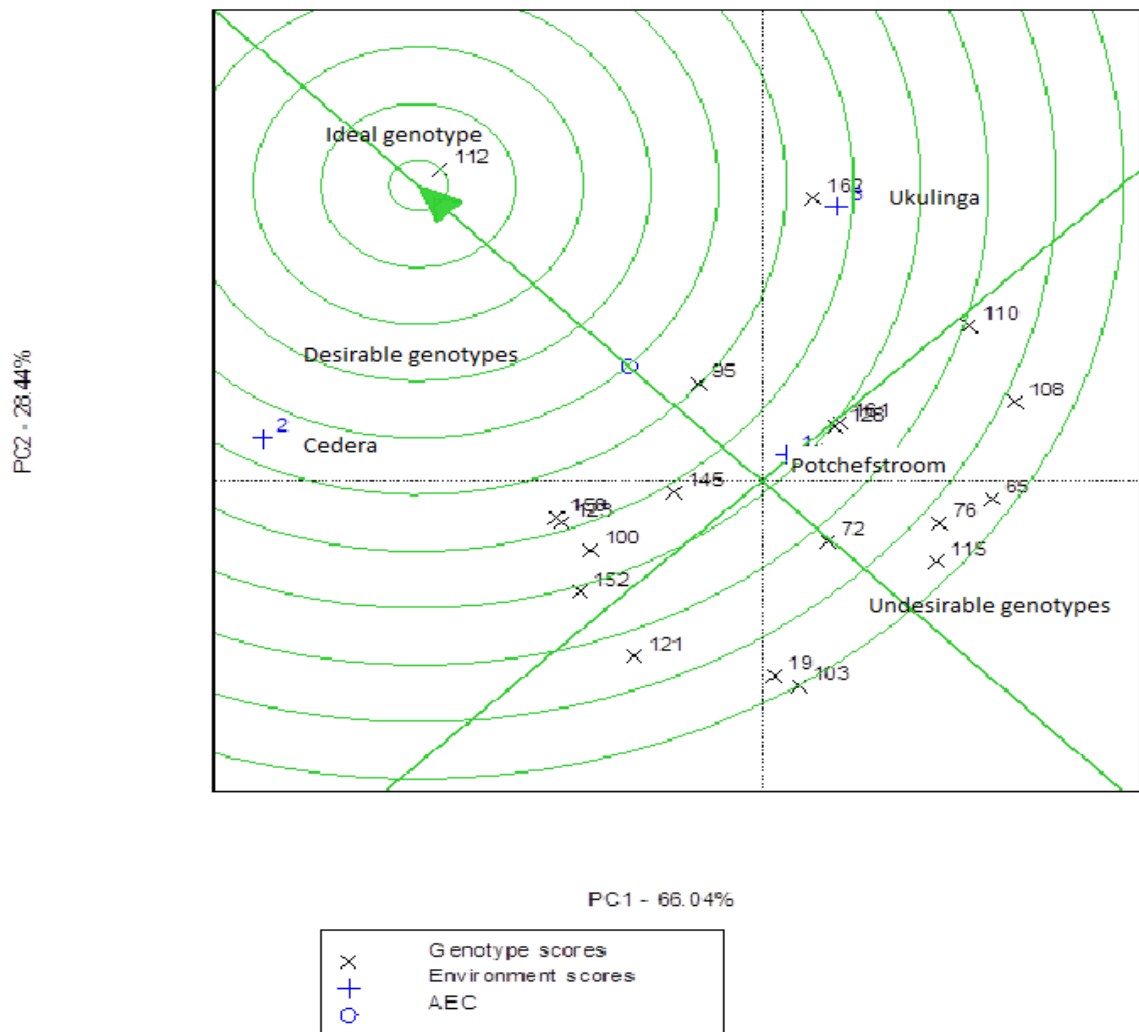
Figure 7. 5: GGE biplot based on genotype-focused scaling for comparison of genotypes with ideal genotype for Population B.

Figure 7.6 shows GGE-biplot of PC2 and PC1 based on environment-focused scaling for comparison of the environments with the ideal environment for Population B. Cedara Research Station was classified as the ideal testing environment as it almost fell in the centre of the concentric circles. Ukulinga Research Station was classified as favourable, while Potchefstroom Research Station fell short of being classified as unfavourable testing site for Population B trial.

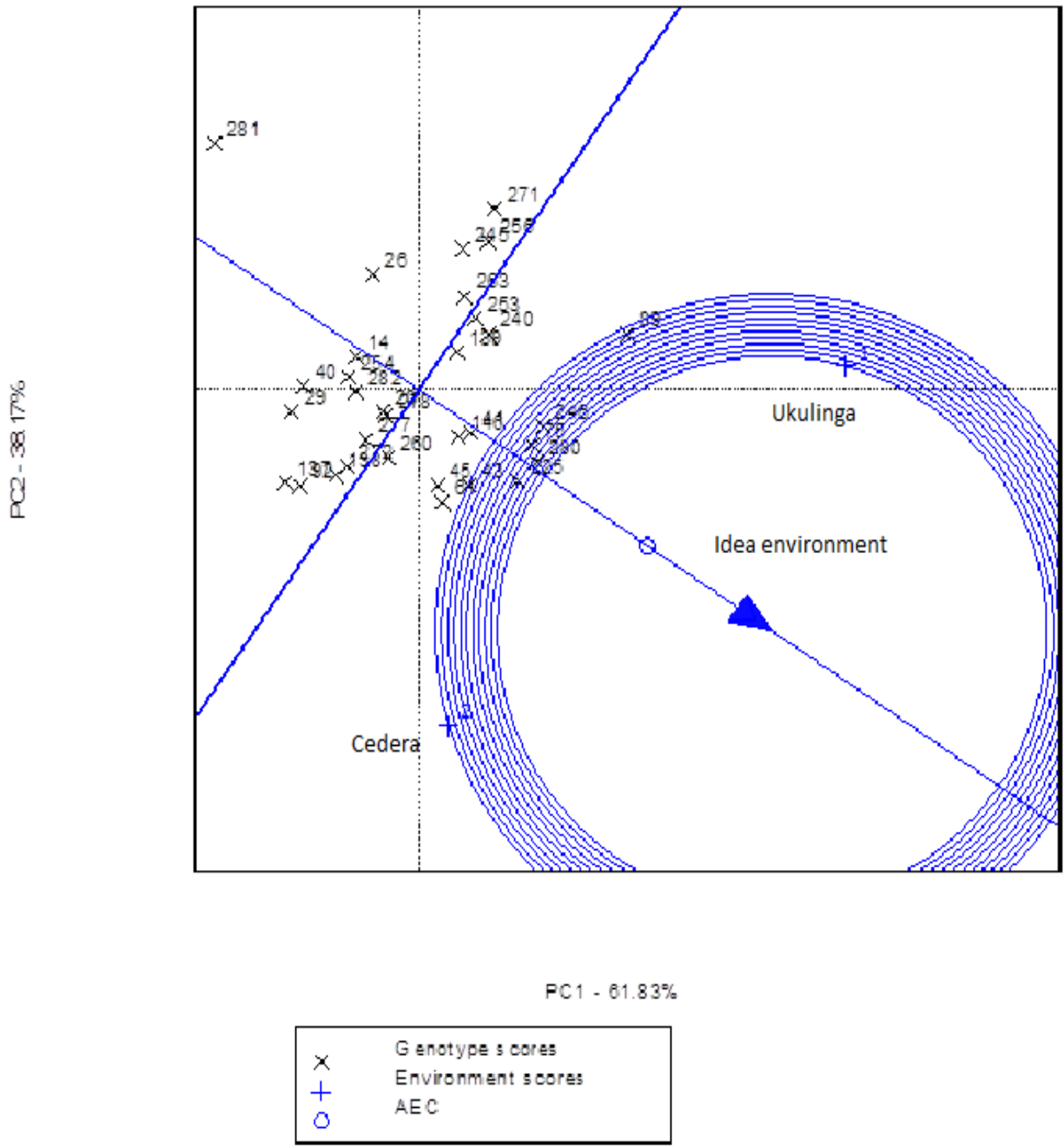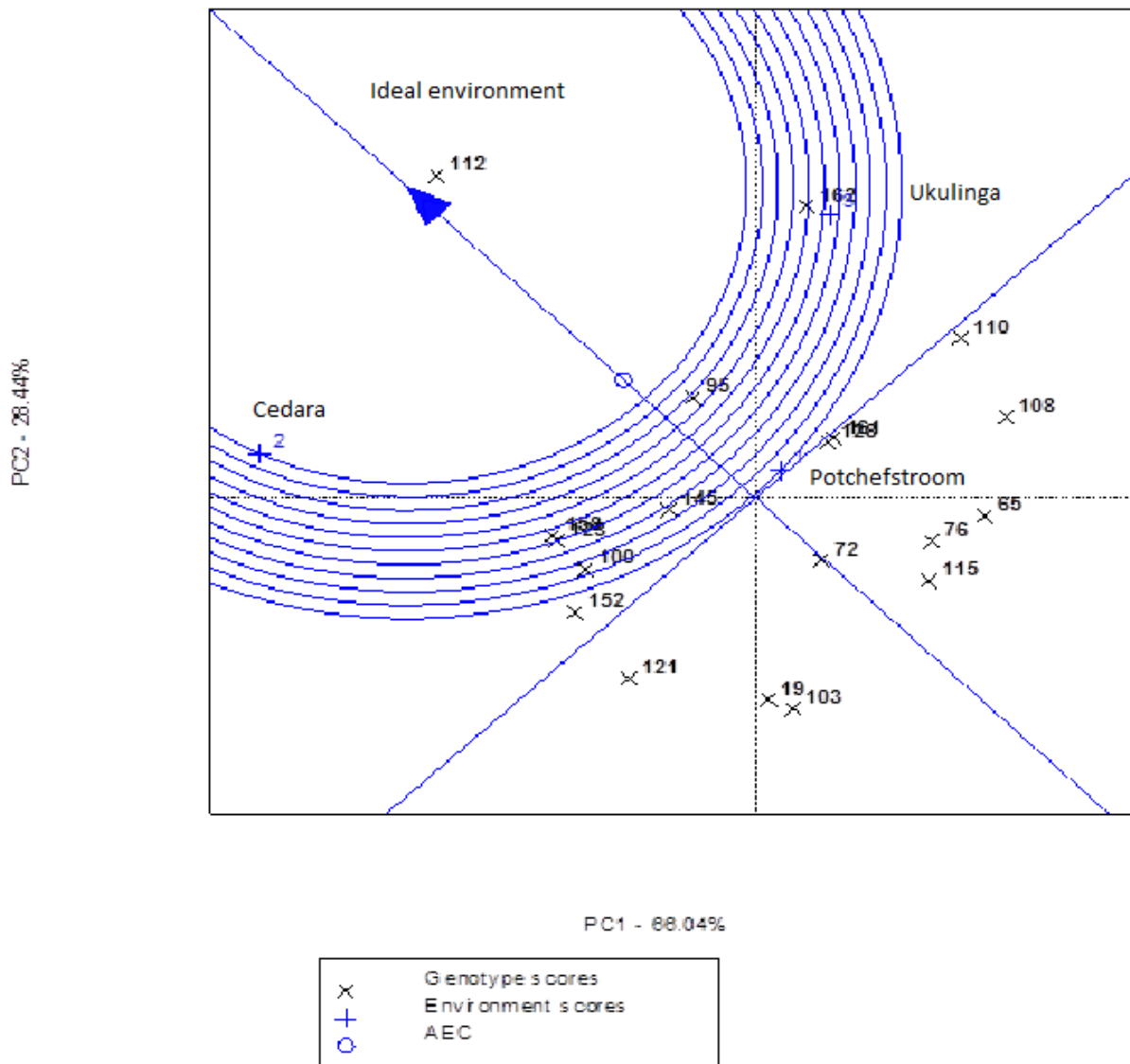**Figure 7. 6: GGE-biplot based on environment-focused scaling for comparison of the environments with the ideal environment for maize hybrid population B. Details of environments are given in Table 5.1.**

7.4   Discussion

7.4.1 GGE-biplots patterns

7.4.1.1   Genotype evaluation based on GGE-biplots for Population A and B

Genotype, and genotype-by-environment interaction (GGE) biplots (Yan et al., 2000) allows visual examination of the genotype by environment pattern of multi-environment trials (MET) data. In the current study, GGE biplots were plotted for entries in population A and B. Visualization of which-won-where pattern of MET data is important for studying the possible existance of different mega-environments in a region (Gauch and Zobel, 1997; Yan et al., 2000). In Population A MET data, two mega environments were identified with the winner genotypes being 89 (12C20628) generated from introgression inbred line bred at Kadoma Research Centre crossed to T2 tester; and 25 (12C19813) developed from introgression inbred line bred at Rattary Anorld Reseach Station crossed to T1 tester). In Population B MET data also identified two mega environments defined by winner genotypes: being 162 (PAN6Q445B); and 110 (13XH346) generated from introgression line bred at Ukulinga Research Station crossed to T2 tester; and 108 (13XH344) developed from crossing introgression inbred line bred at Ukulinga Research Station and T2 tester. This indicates that in Population A, introgressed lines bred from all 3 environments except Kadoma Research Centre demonstrated out-standing perfomance in hybrid combinations across environments. This demostrates that all the 3 envrionments can be used to breed tropical lines that have high yield potential in hybrid combinations. Outstanding yield perfomance also indicates the effectiveness of introgressing temperate germplasm in improving yield perfomance of tropical germplasm in South African environments. Testers 1 and 2 were combining well with the introgressed lines in producing winner genotypes. Hence these testers can be recommended for test crossing introgressed lines from Population A in future studies. Ukulinga Research Station was able to clearly descriminate entries for Population A and B. An indication that this environment can be effectively used for phenotypic selection of maize hybrids for adaptability to South African environments.

In Population A, entry 89 (12C20628 constituted between tester T2 and introgression line bred at Kadoma Research Centre at Ukulinga Research Station can be recommended for further testing towards commercialisation due to its outstanding performance. While at Cedara all the experimental entries were out competed by check entry (PAN6227). In Population B, entries 110 (13XH346 generated from introgression line bred at Ukulinga Research Station crossed to T2 tester), 112 (13HX349 generated from crossing introgression line bred at Ukulinga Research Station to T2 tester) and 128 (13XH1060 developed from crossing introgression line bred at Ukulinga Research Station and T4 tester) displayed out-standing yield perfomance at Ukulinga, Cedara and Potchefstroom Research Station, respectively. Hence these maize genotypes can be recommended for further testing towards commercialisation. Outstanding genotypes in Population B demonstrated that introgressed lines bred from Ukulinga Research Station were outstanding in hybrid combinations. This indicates that introgression of temperate germplasm was effective in producing tropical germplasm that had outstanding yield perfomance in South Africa warm environments. Testers T2 and T4 combined well with the introgressed lines to produce winner genotypes across environments. Hence these testers can be used for test crossing inbred lines developed from Population A.

7.4.1.2   Ideal genotypes

According to Kaya et al. (2006) and Dehghani et al. 2009, yield potential and stability of genotypes are evaluated by an average environment cordination (AEC) method. In this method, an average environment is defined by the average PC1 and PC2 scores of all the environments. An ideal genotype should have the highest mean yield perfomance and stability across all the environments and may not exist, but can be used as reference for genotype evaluation (Yan and Tinker, 2006). In Population A, four entries: 280 (PAN6Q445B) adapted commercial check; 75 (12C20595) constituted from crossing introgression line bred at Rattray Anorld Research Station and T2 tester; 225 (12C22785) generated from crossing introgression line bred from Rattray Anorld Research Station and T4 tester; and 246 (11C1774) developed from

crossing a temperate line bred from conventional pedigree breeding in South Africa programme to T4 tester were defined to be close to ideal. In Population B, only one entry 112 (13XH349) developed from crossing introgression line bred from Ukulinga research Station to T2 tester was defined as ideal genotype. These genotypes can be used in future similar projects as reference in selecting for maize genotypes that are defined as ideal genotypes in similar future projects.

7.4.1.3   Ideal environment

An ideal testing environement should have the ability to discriminate genotypes in terms of genotypic main effect during evaluation. This environment should have large PC1 scores and small PC2 scores, and may not exist in reality, it can be used as a reference for genotype selection in the multi-location trials (Kaya et al., 2006). In the current study all the three environments were not ideal for the entries tested. They can all be defined as favourable environment that can be used for evaluating high yield potential.

7.5   Conclusion

The following hybrids were identified as stable and adaptable: 89-12C20628, 25-12C19813, 110-13XH346, 112-13XH349, and 108-13XH344. They were comparable to standard commercial hybrid checks. Hybrids 75-12C20595, 225-12C22785, 246-11C1774 and 112-13XH349 were considered as ideal alongside the commercial hybrid check PANQ445B.

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8   An economic appraisal of conventional and "shuttle breeding" programmes: Implications for small and medium enterprises in the seed industry

Abstract

Economic appraisal for agricultural projects is important for investor decision making processes. The study sought to determine economic viability of conventional and "shuttle breeding" programmes for development of new maize hybrid varieties for South African warm temperate environments. The net present value (NPV) was adopted as the cost benefit analysis technique. The costs consisted of maize hybrid research and development, seed production and costs of sales. Revenue was estimated based on a conservative approach of 1-16% market share obtained over six years. A cost of capital of 18% was assumed. The conventional breeding programme in Zimbabwe had a high NPV $1, 779, 084.00 (13%) relative to conventional breeding programme carried out in South Africa; hence it has the potential to increase shareholder value in the long-run. The "shuttle breeding" programme attained a positive NPV of $1, 834, 166.00, indicating an increase in shareholder value through an opportunity cost of $326, 138. 00 (17%) and $55 082.00 (3%) relative to conventional breeding programmes carried out in South Africa and Zimbabwe, respectively. This study concluded that carrying out conventional breeding in South African is expensive compared to conventional breeding carried out in Zimbabwe. Despite the "shuttle breeding" programme having showed a marginal NPV (3%) gain in comparison with conventional breeding programme carried out in Zimbabwe; genetic gains attained makes it a viable option. Therefore, small and medium scale seed companies intending to breed and commercialize in the South African temperate environments may cost effectively achieve this through the use of "shuttle breeding" programmes. It has the advantage of achieving high NPV in the long-run thereby increasing shareholder value.

Keywords: maize, conventional and shuttle breeding, cost benefit analysis, net present value

8.1 Introduction

Maize (*Zea mays*, L) is an important cereal crop that has high economic value for the majority of the South African population. According to the Grain South Africa (2013), importance of maize is extended to its significant position as a net earner of foreign currency to South African economy. Undoubtedly, multinational Corporations (MNCs) such as DuPont, Monsanto and Limagrain appreciate the current value, potential and lucrativeness of this market resulting in increased activity that has weakened competitive advantage of small and medium scale seed companies. Regrettably tropical germplasm acquired from Consultative Group on International Agricultural Research (CGIAR) institutes by the small and medium scale (SMS) seed companies for direct introduction into South African warm temperate environments is characterized by lack of adaptability. There are no similar programmes that generate maize hybrid varieties that would be freely available for the SMS seed companies to market in South Africa. Conventional breeding strategies that can be exploited to generate new varieties for the South African market would be too expensive to be sustained by SMS seed companies. Therefore, there is need to assess alternative breeding strategies available for filling up the vacuum created.

Small and medium scale seed companies have the option to conventionally breed in South Africa or neighbouring countries such as Zimbabwe and then deploy products to South African target environments. Alternatively they can engage in a "shuttle breeding" programme between South Africa and Zimbabwe to reduce cost associated with breeding research. Genetic gains can be realised using both conventional and "Shuttle breeding programme as demonstrated in Chapters 5, 6 and 7, respectively. However, genetic gain in maize hybrid improvement should be complemented with full consideration of cost effectiveness of the programme to ensure long term profitability and sustainability. Small and medium scale seed companies do not have sufficient information regarding potential returns for breeding research in South Africa.

In maize breeding programmes, release of an improved variety requires huge financial and human investment; and tends to be long term. Shimelis and Laing

(2012) attribute this cost to: plant breeding research-'pre-breeding'; actual breeding *per se;* multi-locational evaluation trials; national variety-registration procedures; maintenance and multiplication of candidate varieties for seed production; and marketing of the improved products. Therefore, it is prudent in the current study to carry out an economic appraisal of conventional and "shuttle" breeding programmes and subsequent commercialization of adapted maize hybrid variety in South African temperate environment.

Establishment of a cost-effective breeding programme ensures long term sustainability of the programme. The study applied cost-benefit analysis (CBA) to perform an economic analysis of breeding programmes. Riley (2012) and Watkins et al. (2013) define CBA as a systematic process for calculating and comparing benefits and costs of a project for two purposes. The first one is to determine if it is a sound investment/decision (justification/feasibility). Secondly to provide an economic basis of comparing the performance of two or more options. In CBA, benefits and costs are expressed in monetary terms, and are adjusted for the time value of money, so that all flows of project cost over time (which tend to occur at different points in time) are expressed on a common basis in terms of their "net present value" (NPV) (Riley, 2012). Net present value (NPV) compares the difference between an investment's market value and its cost taking inflation and returns into account with the goal of creating value for the shareholders (Firer et al., 2008; Okeno et al., 2010). A positive net value indicates a viable project.

The objective of this study was to apply cost benefit analysis to evaluate the financial performance of conventional and "shuttle breeding" programmes in breeding maize hybrids for adaptability and subsequent commercialization in South African warm temperate environments. The knowledge would be crucial for the SMS seed companies that plan to engage in the maize seed business in South Africa.

8.2   Materials and Methods

8.2.1 Alternative breeding strategy

Conventional and "shuttle" breeding programmes as depicted in Figure 8.1 were used to breed tropical germplasm for commercialization of at least one improved variety in South African target environments. The conventional breeding programme had two options; one carried out at Kadoma Research Centre in Zimbabwe. In this option crosses were generated and advanced to F_2 generation in South Africa. The segregating F_2 bulk seed was then advanced to F_3 generation through pedigree selection at Kadoma Research Centre. Second option breeding was carried out at Ukulinga Research Station in South Africa where a complete pedigree selection was undertaken from segregating F_2 to fixed F_7 inbred lines. The "shuttle breeding" programme was carried out as shown in Figure 8.1 involving three distinct selection environments between South Africa and Zimbabwe.
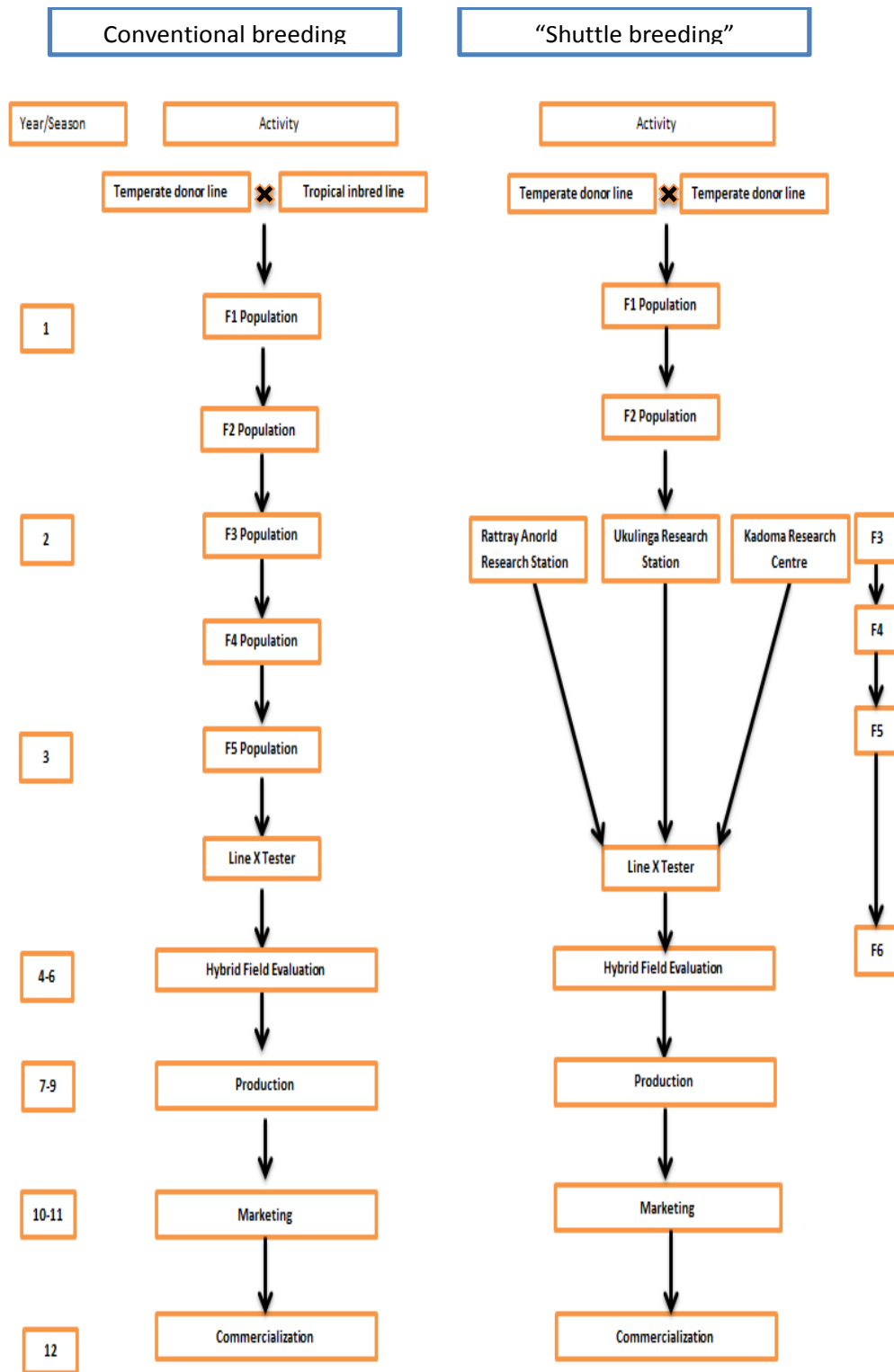
Figure 8. 1: Schematic flow of "shuttle breeding" and conventional breeding programme activities

8.3 Economic analysis

8.3.1 Cost and benefits elements

The CBA approach recommends a precise quantification of project benefits and costs to ensure reliability and consistence of the outputs. The benefits of the breeding programmes were based on the potential revenues from seed sales for the two breeding programmes. Since the hybrid varieties are not commercialised projected market share, yield and price estimates were used to calculate potential long-term benefits of both conventional and shuttle breeding programmes (Table 8.1). The costs of releasing an adapted maize hybrid variety through conventional and "shuttle" breeding programmes (Figure 8.1) were classified as:

1. Research cost
   - line development-starting from identification of parental maize lines, crossing and advancement from F_1-F_6.
   - hybrid development-from creation of test cross hybrids testing crossing through line x tester mating design scheme.
   - hybrid evaluation-this entails extensive multi-locational trials in target environments.
2. Seed production
   - variety registration requirements
   - maintenance and multiplication of variety
3. Marketing
   - cost of sales
   - distribution of variety seed
   - commercialization/release

8.3.2 Market assumptions

Conventional and "shuttle breeding" programmes research costs data in Zimbabwe and South Africa were collected from primary data of actual direct research costs and fixed overheards for the project. The South African market for non-genetically modified white maize is on average 25 000 metric tonnes (South Africa National Seed Organization, 2013). Sales and production cost data is based on a generic business activity on the South African market. All cash flows were nominal cash flows and a discount rate of 18% was used because the cost of the capital was 18%. The study is based on the following production and market assumptions:

- Farmers are conservative hence they gradually take up new hybrids.
- A new improved hybrid will attract 1% of the market and that the market share doubles in each subsequent year for the first four years and then reaches its plateau as depicted in Table 8.1.

Table 8. 1: Market share and seed pricing for improved hybrid variety

Year	Market Share (%)	Seed maize (tonnes)	Price (US$1.50)/kg
1	1	250	375000
2	2	500	750000
3	4	1000	1500000
4	8	2000	3000000
5	16	4000	6000000
6	16	4000	6000000

- A cost of capital of 18% was used to discount benefits and costs (Appendix 8.1) that were subsequently used to calculate net present value
- International investment appraisal was used whereby South African cash flows in Rands were converted at a rate of ZAR10/$1.
- Cash flows were taxed at 10% in South Africa and 25.75% in Zimbabwe assuming that tax treaty existed between the host and parent country. Hence an average tax of 18% was used.

- The research costs incurred during the 6-year research period would not be incurred during the commercialisation phase. The second 6 year period (commercialisation phase) is composed of production costs and no research costs are incurred. Detailed costing of each element for conventional breeding and "shuttle" breeding programmes is shown in Appendix 8.2 to 8.4, respectively. Cash inflows were experienced in the selling phase.

- During the research phase, working capital increases or decreases relative to amount of capital required in each subsequent year. Working capital requirements dependent on: size of nursery, trials and production quantity as indicated by number of rows planted (Appendix 8.2 to 8.4).

8.3.3 Calculations

Net cash flow of each project (Table 8.2, 8.3 and 8.4) was calculated as:

Net cash flow = sales revenue – (production + marketing + research)

Table 8. 2: Project cash flow (Rands) a for conventional maize breeding programme in Zimbabwe

Timeline (years)	0	1	2	3	4	5	6	7	8	9	10	11
Sales		0.00	0.00	0.00	0.00	0.00	3750.00	7500.00	15000.00	30000.00	60000.00	60000.00
Research		(67.62)	(165.63)	(133.89)	(72.94)	(131.53)	0.00	0.00	0.00	0.00	0.00	0.00
Production		0.00	0.00	0.00	0.00	0.00	(173.50)	(346.50)	(693.00)	(1386.00)	(2772.00)	(2772.00)
Marketing		0.00	0.00	0.00	0.00	0.00	(375.00)	(750.00)	(1500.00)	(3000.00)	(6000.00)	(6000.00)
Increase/Decrease in invest		0.00	(98.01)	31.74	60.96	(58.59)	(1602.44)	(3370.50)	(6091.00)	(12682.00)	(25364.00)	(25364.00)
Tax @ (10 %)		0.00	0.00	0.00	0.00	0.00	(375.00)	(750.00)	(1500.00)	(3000.00)	(6000.00)	(6000.00)
total cost		(67.62)	(263.64)	(102.15)	(11.98)	(190.12)	(2150.94)	4467.00	(8284.00)	(17068.00)	(34136.00)	(34136.00)
Cash flow		(67.62)	(263.64)	(102.15)	(11.98)	(190.12)	1599.06	3033.00	6716.00	12932.00	25864.00	25864.00

Figures are in thousand (X1000); Year 0- initial year of investment; Inc/Dec in invest-means Increase/decrease in capital investment

Table 8. 3: Project cash flow (Rands) for a conventional maize breeding programme in South Africa

Timeline (years)	0	1	2	3	4	5	6	7	8	9	10	11
Sales	0.00	0.00	0.00	0.00	0.00	0.00	3750.00	7500.00	15000.00	30000.00	60000.00	60000.00
Research	(421.32)	(455.95)	(495.83)	(327.23)	(376.5)	(299.07)	0.00	0.00	0.00	0.00	0.00	0.00
Production	0.00	0.00	0.00	0.00	0.00	0.00	(173.5)	(346.50)	(693.00)	(1386.00)	(2772.00)	(2772.00)
Marketing	0.00	0.00	0.00	0.00	0.00	0.00	(375.00)	(750.00)	(1500.00)	(3000.00)	(6000.00)	(6000.00)
Inc/Dec in invest	0.00	25.38	(39.89)	168.6	249.49	(17.42)	(1546.98)	(2713.50)	(4777.00)	(10054.00)	(25364.00)	(25364.00)
Tax @ 10%)	0.00	0.00	0.00	0.00	0.00	0.00	375.00	(750.00)	(1500.00)	(3000.00)	(6000.00)	(6000.00)
Total Costs	(421.32)	(430.57)	(535.72)	(158.63)	(127.01)	(316.49)	2095.48	(3810.00)	(6970.00)	(14440.00)	(34136.00)	(34136.00)
Cash flow	(421.32)	(430.57)	(535.72)	(158.63)	(127.01)	(316.49)	1654.52	3690.00	8030.00	15560.00	25864.00	25864.00

Figures are in thousand (X1000); Year 0- initial year of investment; Inc/Dec in invest-means Increase/decrease in capital investment

Table 8. 4: Project cash flow (Rands) for a "shuttle" maize breeding programme

Timeline (years)	0	1	2	3	4	5	6	7	8	9	10	11
Sales	0.00	0.00	0.00	0.00	0.00	0.00	3750.00	7500.00	15000.00	30000.00	60000.00	60000.00
Research	(421.32)	(81.25)	(212.87)	165.40	227.12	299.07	0.00	0.00	0.00	0.00	0.00	0.00
Production	0.00	0.00	0.00	0.00	0.00	0.00	(173.25)	(346.50)	(693.00)	(1386.00)	(2772.00)	(2772.00)
Marketing	0.00	0.00	0.00	0.00	0.00	0.00	(375.00)	(750.00)	(1500.00)	(3000.00)	(6000.00)	(6000.00)
Inc/Dec in invest	0.00	(177.58)	(131.62)	47.48	(61.72)	(71.96)	(1853.53)	(3370.50)	(6091.00)	(12682.00)	(2536.40)	(25364.00)
Tax @(10%)	0.00	0.00	0.00	0.00	0.00	0.00	(375.00)	(750.00)	(1500.00)	(3000.00)	(6000.00)	(6000.00)
Total Cost	(421.32)	(258.83)	(344.49)	117.92	(288.84)	(371.03)	(2401.78)	(4467.00)	(8284.00)	(17068.00)	(11308.40)	(34136.00)
Cash flow	(421.32)	(258.83)	(344.49)	(117.92)	(288.84)	(371.03)	1348.22	3033.00	6716.00	12932.00	48691.60	25864.00

Figures are in thousand (X1000); Year 0- initial year of investment; Inc/Dec in invest-Increase/decrease in capital investment.

Detailed costing of each element for conventional breeding and "shuttle" breeding programmes is shown in Appendix 8.2 to 8.4, respectively.

In determining the economic benefits, an assumption was made that at least one adapted and productive hybrid was commercialized in South African warm temperate environments for both breeding strategies. Economic analysis was based on spreadsheet budgets using actual costs (investment) adjusted for time value, so that all flows of benefits and costs over time were expressed on a common basis in terms of their "net present value" (Riley, 2012). Net present value (NPV) was the evaluation criterion carried out for economic analysis of the current study. The NPV for each breeding programme was estimated as;

$$
\text{NPV} = \sum_{t=0}^{T} \left[\frac{[N \ \sum_{i=1}^{T} ER_1]\mathbf{t}}{(1+r)^t} - C_t \right]
$$

Where: T is the evaluation period in years, $\sum_{i=1}^{T} ER_1$ is summation of cash flows from $t_{=0\ldots11}$, r the discount rate (18%), t is time in years, C_t is the initial cash flows at t =0

The net present value technique was the investment appraisal technique used to check long-term sustainability for both conventional and shuttle breeding programmes. The net present value of all the research phase cash flows, production and marketing phase cash flows was calculated over a period of 12 years. A formal selection criterion for the net present value measure of project worth is to accept all independent projects with zero or greater NPV (Gittinger, 1982). A positive NPV means that the investment is worth to investors, while a negative NPV would have a case in which the present worth of the benefits is less than the present worth of the cost stream. Under such circumstances the investors might need to invest in more lucrative projects.

8.4   Results

8.4.1 Conventional breeding

Investment appraisal for the conventional breeding programme carried out in Zimbabwe indicated a positive NPV of $1, 779, 084. 00 (Table 8.5). Investment costs in this breeding programme were paid off in the sixth year, which represented a single year after commercial production. Similarly, conventional breeding programme carried out in South Africa showed a positive NPV $1, 508, 208.00, while investment costs were paid off within two years of commercial production (that is in seventh year). Adopting conventional breeding in Zimbabwe ahead of South Africa would marginally improve shareholder value by 13% ($1, 508, 208.00) to $1, 779, 084. 00).

Table 8. 5: Conventional breeding programmes net present values (US dollars)

		Conventional breeding in Zimbabwe			Conventional breeding in South Africa		
Timeline (years)	DF†	Cash flow	Present values	Net present value	Cash flow	Present value	Net present value
0	1				(42132)	(42132)	(42132)
1	0.85	(6762)	(6762)	(6762)	(43057)	(36598)	(78730.5)
2	0.72	(26364)	(22409)	(29171)	(53572)	(38572)	(117302)
3	0.61	(10215)	(7355)	(36526)	(15863)	(9676)	(126979)
4	0.52	(1198)	(731)	(37257)	(12701)	(6605)	(133583)
5	0.44	(19012)	(9886)	(47143)	(31649)	(13926)	(147509)
6	0.37	159906	70359	23215	165452	61217	(86291.6)
7	0.31	303300	112221	135436	369000	114390	28098.44
8	0.27	671600	208196	343632	803000	216810	244908.4
9	0.23	1293200	349164	692796	1556000	357880	602788.4
10	0.19	2586400	594872	1287668	2586400	491416	1094204
11	0.16	2586400	491416	1779084	2586400	413824	1508028

† Discount factor at 18%;

8.4.2 Shuttle breeding

The NPV for "shuttle breeding" programme option is positive ($1, 834, 166. 00) (Table 8.6). Similar to conventional breeding in South Africa, investment cost in the "shuttle breeding" programme is paid off within the initial two years of commercial production (seventh year). The "shuttle breeding" programme gave shareholders a value addition that was 17% and 3% higher than the conventional breeding programmes carried out in South Africa and Zimbabwe, respectively.

Table 8. 6: "Shuttle" maize breeding programme net present value (US dollars)

Timeline (years)	† Discount Factor	Cash flow	Present Value	Net present value
0	1	(42132)	(42132)	(42132)
1	0.85	(25883)	(22001)	(64133)
2	0.72	(34449)	(24803)	(88936)
3	0.61	(11792)	(7193)	(96129)
4	0.52	(28884)	(15020)	(111149)
5	0.44	(37103)	(16325)	(127474)
6	0.37	134822	49884	(77590)
7	0.31	303300	94023	16433
8	0.27	671600	181332	197765
9	0.23	1293200	297436	495201
10	0.19	4869160	925140	1420342
11	0.16	2586400	413824	1834166

† Discount factor at 18%

8.5   Discussion

8.5.1   Conventional breeding options in Zimbabwe and South Africa

The Conventional breeding option carried out in Zimbabwe with an arbitrage market in South Africa produced the highest net present value of $ 1, 779, 084 .00. This meant that investing in conventional breeding in Zimbabwe with a marketing option in South Africa would increase shareholder value by $207 876 .00 (13%) relative to use of the conventional breeding programme in South Africa. This indicates that the cost of breeding in Zimbabwe is low (221%) and this may be attributed to availability of abundant semi-skilled to skilled low cost human labour; and own research facilities. Winning genotypes were also realised from this conventional breeding programme (see Chapter 5, 6 and 7) an indication that there would not be genetic gain penalty in using this cost efficient breeding strategy. Lower NPV attained in the conventional breeding programme carried out in South Africa may be attributed to high (68%) cost of human labour for both skilled and unskilled labour; crop husbandry practices such as herbicide and pesticide usage; and out-sourced research facilities. Despite a low NPV, the conventional breeding programme carried out in South Africa also had the majority of the winning genotypes (see Chapter 5, 6 and 7), hence there is need for a trade-off between increasing shareholder value and release of competitive maize hybrid varieties for the South African market. The genetic gain is likely to be greater when crop varieties are bred in the environment of potential deployment.

Conventional breeding programme carried out in Zimbabwe achieved positive NPV; positive gross margins in the sixth year from initial year of investment compared to the seventh year observed in the conventional breeding programme carried out in South Africa. This indicates that initial investment costs in the conventional breeding option in Zimbabwe were paid off earlier (one year) relative to the South African conventional breeding option. Investment in conventional breeding programme in Zimbabwe is anticipated to bring positive returns in a shorter time (1 year of commercial production) relative to conventional breeding programme in South Africa (2 years of commercial production). Nevertheless, all the NPVs indicated positive

values, thus indicating that the investment meets the corporate objective of increasing shareholder value.

8.5.2   Cost and market arbitrage opportunities in breeding cycle

Since the selling phase cash flows were the same in all options, conventional breeding programme in South Africa could be regarded as more costly and shareholder value erosive when compared to the conventional breeding option in Zimbabwe. Arbitrage exists where there are cost differences between two or more markets that allow profit to be realised at minimum risk (Brown, 2008). It was observed that there existed arbitrage in research costs between Zimbabwe and South Africa. Research factor conditions namely labour, consumables, and crop husbandry practices in South Africa (Table 8.2) were more costly (68%) which resulted in the conventional breeding option in Zimbabwe having a competitive advantage. The presence of owned research infrastructure in the form of irrigation system, research equipment among other factors, which was experienced for the conventional breeding options carried out in Zimbabwe relative to out-sourced research infrastructure and personnel in South Africa, could also have caused the vast differences in research costs between conventional breeding programmes in Zimbabwe and South Africa. It could mean that the costs of out-sourcing research equipment and personnel in South Africa, reduced shareholder value as shown by a low NPV. Cost arbitrage existed in Zimbabwe as depicted in conventional breeding costs in Zimbabwe, while market arbitrage existed in South Africa for the seed product.

8.5.3   "Shuttle breeding" programme

The results indicate that if the appropriate genetic base population is identified, cost efficient breeding of maize hybrid varieties that are adaptable to South African environments can be achieved using a "shuttle breeding" programme. Labour cost (skilled and unskilled) and research facilities are relatively cheap in Zimbabwe, hence shareholder value can be increased by carrying out most of the breeding process in Zimbabwe. The NPV for "shuttle breeding" programme achieved a low gain of (3%)

over conventional breeding in Zimbabwe. There might be need to further increase the NPV by increasing the number of breeding generations carried out in Zimbabwe and reduce trial sites during muti-locational field evaluation in South Africa. This can also be coupled by carrying out seed production in Zimbabwe instead of South Africa. However, genetic gains achieved in the resultant inbred lines and hybrids from the "shuttle breeding" programme (see Chapter 5, 6 and 7) indicate that it is a cost efficient breeding strategy that ensure release of competitive products.

8.6 Implications for breeding programme

High cost of setting up and operating a breeding programme in South Africa indicates that small and medium scale seed companies operating outside South Africa, in particular Zimbabwe need to establish a cost effective breeding strategy. Use of a "shuttle breeding" programme may provide a viable breeding option for these programmes that are intending to develop germplasm that is adaptable to South Africa warm temperate environments. However, there is need to identify suitable base populations for breeding with emphasis on traits that enhance adaptability, early physiological maturity aspects (flowering days and low grain moisture content at harvest), better standing ability and high grain yield. Pedigree crosses F_1 can be carried out in Zimbabwe and this can also include progeny advancement from F_1 to F_3, without any major selection being carried out. Resultant F_3 families can be evaluated in South Africa where major selection of the desired economic traits is carried out. Parallel to F_3 evaluation will be the early generation test crossing of these families through line x tester analysis in Zimbabwe. Single cross hybrids generated from the test crosses will then be evaluated in South Africa for possible release. Advancement of progenies from F_4 to F_6 will be based on test cross evaluation results. Inbred lines that have good combining ability can be fixed through the use of option of double haploid technique to ensure efficiency. Cost opportunity is realised by carrying out all the non-critical stages of research in Zimbabwe where research costs is lower. However, it should be noted that the economic climate in both environments can change over time hence the need to continuously monitor economic trends to ensure relevance of the breeding programmes.

8.7   CONCLUSION

The research results revealed that carrying out conventional breeding in South Africa is expensive relative to conventional breeding carried out in Zimbabwe. This can be attributed to arbitrage cost conditions between Zimbabwe and South Africa and purchasing power parity conditions. It can be concluded that it would erode shareholder value to carry out conventional breeding in South Africa and market in South Africa in relation to the option of carrying out conventional breeding in Zimbabwe and marketing in South Africa. Despite the "shuttle breeding" programme having a marginal gain in NPV (3%) than the conventional breeding programme in Zimbabwe, it is a viable and sustainable option as indicated by the genetic gains attained (see Chapter 5, 6 and7). In the long run the "shuttle breeding" programme has the highest NPV, and had the advantage of increasing shareholder value relative to the conventional breeding programmes. However it is also important to note that economic conditions may change in Zimbabwe resulting in increased operating costs to comparable levels as South Africa; thus affecting the costs of the "shuttle breeding" programme. Therefore, there is need to continuously monitor market trends to ensure relevance of the programme.

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Appendices

Appendix 8.1: Discount factor (per year) for a range of discount rates

	Discount rate								
Number of periods	2%	4%	6%	8%	10%	12%	14%	16%	18%
1	0.9804	0.9615	0.9434	0.9259	0.9091	0.8929	0.8772	0.8621	0.8475
2	0.9612	0.9246	0.89	0.8573	0.8264	0.7972	0.7695	0.7432	0.7182
3	0.9423	0.889	0.8396	0.7938	0.7513	0.7118	0.675	0.6407	0.06086
4	0.9238	0.8548	0.7921	0.735	0.683	0.6355	0.5921	0.5523	0.5158
5	0.9057	0.8219	0.7473	0.6806	0.6209	0.5674	0.5194	0.4761	0.4371
6	0.888	0.7903	0.705	0.6302	0.5645	0.5066	0.4556	0.4104	0.3704
7	0.8706	0.7599	0.6651	0.5835	0.5132	0.4523	0.3996	0.3538	0.3139
8	0.8535	0.7307	0.6274	0.5403	0.4665	0.4039	0.3506	0.305	0.266
9	0.8368	0.7026	0.5919	0.5002	0.4241	0.3606	0.3075	0.263	0.2255
10	0.8203	0.6756	0.5584	0.4632	0.3855	0.322	0.2697	0.2267	0.1911
11	0.8043	0.6496	0.5268	0.4289	0.3505	0.2875	0.2366	0.1954	0.1619
12	0.7885	0.6246	0.497	0.3971	0.3186	0.2567	0.2076	0.1685	0.1373
13	0.773	0.6006	0.4688	0.3677	0.2897	0.2292	0.1821	0.1452	0.1163
14	0.7579	0.5775	0.4423	0.3405	0.2633	0.2046	0.1597	0.1252	0.0985
15	0.743	0.5553	0.4173	0.3152	0.2394	0.1827	0.1401	0.1079	0.0835
16	0.7284	0.5339	0.3936	0.2919	0.2176	0.1631	0.1229	0.093	0.0708
17	0.7142	0.5134	0.3714	0.2703	0.1978	0.1456	0.1078	0.0802	0.06
18	0.7002	0.4936	0.3503	0.2502	0.1799	0.13	0.0946	0.0691	0.0508
19	0.6864	0.4746	0.3305	0.2317	0.1635	0.1161	0.0829	0.0596	0.0431
20	0.673	0.4564	0.3118	0.2145	0.1486	0.1037	0.0728	0.0514	0.0365

(Adapted from Firer et al. 2008)

Appendix 8.2: Convectional breeding and product life cycle cash flow (US$) in Zimbabwe

Timeline (years)	0	1	2	3	4	5	6	7	8	9	10	11
Number of rows		68	500	276	186	367	1753	19250	38500	77000	154000	154000
Revenue		0.00	0.00	0.00	0.00	0.00	375.00	750.00	1500.00	3000.00	6000.00	6000.00
Production costs @R180/row		0.00	0.00	0.00	0.00	0.00	(17.35)	(346.50)	(693.00)	(1386.00)	(2772.00)	(2772.00)
Gross profit		0.00	0.00	0.00	0.00	0.00	201.75	403.50	807.00	1614.00	3228.00	3228.00
<u>Research costs</u>		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Crop husbandry costs @R100/row		(6.80)	(50.00)	(27.60)	(18.60)	(35.70)	0.00	0.00	0.00	0.00	0.00	0.00
Pollinations		(6.23)	(22.11)	(16.17)	(14.96)	(6.42)	0.00	0.00	0.00	0.00	0.00	0.00
Regular staff		(25.34)	(50.69)	(50.69)	(16.90)	(10.56)	0.00	0.00	0.00	0.00	0.00	0.00
Transport		(6.00)	(9.09)	(9.09)	(4.00)	(18.80)	0.00	0.00	0.00	0.00	0.00	0.00
Accommodation + Subsistence		(16.00)	(16.00)	(16.00)	(10.67)	(5.33)	0.00	0.00	0.00	0.00	0.00	0.00
Research levy @17.5 %		(7.25)	(17.75)	(14.35)	(7.81)	(54.71)	0.00	0.00	0.00	0.00	0.00	0.00
Total Group research costs		(67.62)	(165.63)	(133.89)	(72.94)	(131.53)	0.00	0.00	0.00	0.00	0.00	0.00
Admin,Marketing & Distribution costs		0.00	0.00	0.00	0.00	0.00	(37.50)	(75.00)	(150.00)	(300.00)	(600.00)	(600.00)
PBIT		(67.62)	(165.63)	(33.89)	(72.94)	(131.53)	164.25	328.50	657.00	(1314.00)	2628.00	2628.00
Tax @10%		0.00	0.00	0.00	0.00	0.00	(16.43)	(32.85)	(65.70)	(131.40)	(262.80)	(262.80)
Tax loss		0.00	0.00	0.00	0.00	0.00	(5.72)	0.00	0.00	0.00	0.00	0.00
Foreign cash flows		(67.62)	(165.63)	(133.89)	(72.94)	(131.53)	186.40	361.35	722.70	1445.40	(2890.80)	(2890.80)
Initial investment		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Increase in working capital	(67.62)	0.00	(98.01)	31.74	60.95	(58.59)	(261.47)	(243.00)	(113.60)	(177.20)	(354.40)	(354.40)
Net Foreign cash flows	(67.62)	(67.62)	(263.64)	(102.15)	(11.99)	(190.12)	160.44	303.05	671.60	1293.20	2586.40	2586.40
Exchange rate @10 Rands/$1	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
<u>Home cash flows</u>	(6.76)	(6.76)	(26.36)	(10.21)	(1.20)	(19.01)	16.04	33.71	(60.91)	126.82	253.64	253.64
Management fees/Group overheards	0.00	6.76	16.56	13.39	7.29	13.15	0.00	0.00	0.00	0.00	0.00	0.00
Tax @25.75% Management fees	0.00	0.00	(1.74)	(4.26)	(3.45)	(1.88)	(3.39)	0.00	0.00	0.00	0.00	0.00
Tax @(25.75-10)% remitted profits	0.00	1.07	0.00	0.00	0.00	0.00	0.00	(2.54)	(5.31)	(9.593)	(3.35)	(3.35)

Figures are in thousand (X1000) except for number of rows

Appendix 8.3: Convectional breeding and product life cycle cash flow (US$ dollars) in South Africa

Year	0	1	2	3	4	5	6	7	8	9	10	11
Number of rows	17	188	385				1753	19250	38500	77000	154000	154000
Revenue	0.00	0.00	0.00	0.00	0.00	0.00	375.00	750.00	1500.00	3000.00	6000.00	6000.00
Production costs @ R180/row	0.00	0.00	0.00	0.00	0.00	0.00	(17.35)	(346.50)	(693.00)	(1386.00)	(2772.00)	(2772.00)
Gross profit	0.00	0.00	0.00	0.00	0.00	0.00	201.75	403.50	807.00	1614.00	3228.00	3228.00
Research costs	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Crop husbandry costs @R150.60/row	0.27	0.24	6.01	4.10	4.31	2.31	0.00	0.00	0.00	0.00	0.00	0.00
Pollinations	4.05	4.23	8.64	4.46	4.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Regular staff	24.00	23.50	24.00	16.00	80.00	10.00	0.00	0.00	0.00	0.00	0.00	0.00
Transport	4.77	4.27	4.77	2.37	4.40	1.37	0.00	0.00	0.00	0.00	0.00	0.00
Accommodation + Subsistence	2.77	2.27	2.77	9.23	1.85	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Research levy @17.5 %	6.27	6.29	7.39	4.87	7.58	4.45	0.00	0.00	0.00	0.00	0.00	0.00
Total research costs	42.13	43.06	53.57	32.72	37.65	29.90	0.00	0.00	0.00	0.00	0.00	0.00
Admin,Marketing & Distribution costs	0.00	0.00	0.00	0.00	0.00	0.00	(37.50)	(75.00)	(150.00)	(300.00)	(600.00)	(600.00)
PBIT	(42.13)	(43.06)	(53.57)	(32.72)	(37.65)	(29.91)	164.25	328.50	657.00	(1314.00)	2628.00	2628.00
Tax @10%	0.00	0.00	0.00	0.00	0.00	0.00	(16.43)	(32.85)	(65.70)	(131.40)	(262.80)	(262.80)
Tax loss	0.00	0.00	0.00	0.00	0.00	0.00	(5.72)	0.00	0.00	0.00	0.00	0.00
Foreign cash flows	(42.13)	(43.06)	(53.57)	(32.72)	(37.65)	(24.45)	186.40	361.35	722.70	1445.40	2890.80	2890.80
Initial investment	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Increase/decrease in working capital	0.00	(0.00)	(0.00)	16.86	24.95	(17.42)	(20.96)	(-7.70)	(113.60)	(177.20)	(354.40)	(354.40)
Net Foreign cash flows	(42.13)	(43.06)	(53.57)	(15.86)	(12.70)	(31.65)	165.44	369.05	803.60	155.40	2586.40	2586.40
Exchange rate @10 Rands/$1	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Home cash flows	(42.13)	(43.57)	(53.72)	(15.86)	(12.70)	(31.69)	16.54	36.91	80.30	15.55	25.86	25.86
Tax @(25.75-10)% remitted profits	0.00	0.00	0.00	0.00	0.00	0.00	0.00	(2.68)	(4.66)	(1.31)	(2.35)	(2.35)

Figures are in thousand (X1000) except for number of rows

Appendix 8.4: Shuttle breeding and product life cycle cash flow (US$ dollars)

Year	0	1	2	3	4	5	6	7	8	9	10	11
Number of rows	17	1350	757	439	276	1480	1733	1925	38500	7700	154000	154000
Revenue	0.00	0.00	0.00	0.00	0.00	0.00	375.00	750.00	1500.00	3000.00	6000.00	6000.00
Production costs @R180/row	0.00	0.00	0.00	0.00	0.00	0.00	(173.25)	(346.50)	(693.00)	(1386.00)	(2772.00)	(2772.00)
Gross profit	0.00	0.00	0.00	0.00	0.00	0.00	201.75	403.50	807.00	1614.00	3228.00	3228.00
<u>Research costs</u>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Crop husbandry costs	(2.65)	(13.50)	(75.70)	(43.90)	(43.06)	(230.88)	0.00	0.00	0.00	0.00	0.00	0.00
Pollinations	(40.54)	(8.00)	(28.92)	(20.49)	(27.77)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Regular staff	(24.00)	(25.34)	(50.69)	(50.69)	(80.00)	(10.00)	0.00	0.00	0.00	0.00	0.00	0.00
Transport	(4.77)	(6.00)	(9.09)	(9.09)	(24.02)	(13.65)	0.00	0.00	0.00	0.00	0.00	0.00
Accommodation + Subsistence	(2.77)	(1.60)	(1.60)	(1.60)	(1.85)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Research levy @17.5 %	(6.27)	(1.24)	(32.47)	(25.23)	(33.83)	(44.54)	0.00	0.00	0.00	0.00	0.00	0.00
Total Group research overheards	(42.13)	(81.25)	(212.87)	(165.40)	(227.12)	(299.07)	0.00	0.00	0.00	0.00	0.00	0.00
Admin,Marketing & Distribution costs	0.00	0.00	0.00	0.00	0.00	0.00	(37.50)	(75.00)	(150.00)	(300.00)	(600.00)	(600.00)
PBIT	(42.13)	(81.25)	(212.87)	(165.40)	(227.12)	(299.07)	164.25	328.50	657.00	1314.00	2628.00	2628.00
Tax @10%	0.00	0.00	0.00	0.00	0.00	0.00	(16.43)	(32.85)	(65.70)	(131.40)	(262.80)	(262.80)
Tax loss	0.00	0.00	0.00	0.00	0.00	0.00	(14.07)	0.00	0.00	0.00	0.00	0.00
Foreign cash flows	(42.13)	(81.25)	(212.87)	(165.40)	(227.12)	(299.07)	194.75	361.35	722.70	1445.40	2890.80	2890.80
Initial investment	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tax saving on capital allowances	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Increase/decrease in working capital	0.00	340.07	(131.62)	47.48	(61.72)	(71.96)	(60.61)	(58.35)	(51.10)	(152.20)	(1978.80)	(304.40)
Total	(42.13)	(258.83)	(344.49)	(117.92)	(288.84)	(371.03)	185.35	3303.00	6716.00	1293.20	48696.00	25864.00
Exchange rate @10 Rands/$1	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
<u>Home cash flows</u>	(42.13)	(25.88)	(34.45)	(11.79)	(28.88)	(37.10)	134.83	303.30	671.60	1293.20	4869.60	2586.40
Management fees/Group overheards	0.00	8.12	21.29	16.54	22.71	29.91	0.00	0.00	0.00	0.00	0.00	0.00
Tax @25.75% Management fees		0.00	(2.09)	(5.48)	(4.26)	(5.85)	(34.70)	0.00	0.00	0.00	0.00	0.00
Tax @(25.75-10)% remitted profits		0.00	0.00	0.00	0.00	0.00	0.00	(79.19)	(172.93)	(295.93)	(1251.69)	(635.69)

Figures are in thousand (X1000) except for number of rows

9 Overview of research findings

9.1 Introduction

Tropical maize germplasm that is directly introduced into temperate environments is often characterized by a lack of adaptability, namely: late flowering, excessive rank growth and lodging, high grain moisture content at harvest, and low grain yield potential. Breeding programmes have explored the potential of utilizing exotic genes in tropical and temperate germplasm to enhance adaptability in target environments. Introgression of temperate germplasm into tropical elite maize inbred lines was carried out to enhance adaptability of tropical germplasm to South African warm temperate environments. This chapter summaries findings on the potential of using temperate germplasm in enhancing adaptability of tropical elite inbred lines to South African warm temperate environments. The study objectives are reviewed and a summary of the findings and implications of the findings follow. Recommendations made based on the findings of the study are also reviewed in this chapter.

9.2 Research hypothesis

The following hypotheses were tested:

1. introgression of temperate germplasm in tropical elite inbred lines and selection environment altered genetic diversity and heterotic patterns of introgressed lines,
2. there is a large genetic variation, heritability and correlation of association of desired economic traits among introgressed lines that can be explored in breeding programmes,
3. there is a large genetic gain and introgressed inbred line performance *per se* that can be identified and exploited,

4. high genetic gain and introgressed lines' performance *inter se* can be found in single cross hybrids that are adaptable to South African warm temperate environments,

5. experimental single cross hybrids are comparable to commercial checks and adapted to South African warm temperate environments, and

6. a "shuttle breeding" programme is cost effective for identification of adaptable tropical germplasm for South African warm temperate environments.

9.3   Summary of the main findings

9.3.1 Molecular characterization of maize introgressed lines

A total of 123 inbred lines comprising 76 introgressed lines derived from introgression of temperate germplasm into tropical elite inbred lines, and four generations of pedigree selection in three distinct environments in Zimbabwe and South Africa, 21 tropical elite inbred lines and 26 temperate inbred lines that included the temperate donor inbred line were characterized using 20 SSR markers.

- Twenty SSR markers were used to discriminate the introgressed lines based on their genetic distance and probable heterotic groupings.

- A total of 83 alleles were detected with an average of 4.15 alleles per locus, while allelic diversity was 0.53 and PIC value was 0.47.

- Introgression of temperate germplasm in tropical elite inbred lines did not disrupt heterotic groupings as introgressed lines were inclined towards the original heterotic groups from which they were derived.

- Fourteen per cent of the introgressed lines did not show any inclination to known heterotic clusters.

- Genetic diversity was also identified among introgressed lines developed in the same environment.

- Selection environment did not influence heterotic grouping of introgressed lines.

9.3.2 Genetic variation and relationships between traits

- The study evaluated introgressed maize inbred lines bred from three distinct environments for selected economic traits and grain yield.

- Significant (P<0.05) genetic variation was observed for the selected economic traits and grain yield among the inbred lines within and across sets.

- Heritability estimates ranged from low (21%) to high (91%) for stalk lodging and silking days, respectively.

- Comparison of means of grain yield of introgressed inbred line sets showed that selection environmental had an effect on grain yield.

- Across introgressed inbred line sets, grain yield and ear prolificacy performance showed differences among introgressed lines sets.

- Significant (P<0.05) correlations for grain yield and ear prolificacy were observed among selection environments.

- Positive significant (P<0.05) correlation was detected for grain yield with plant and ear aspects, plant height, root and stalk lodging and grain yield components; ear prolificacy and grain moisture content at harvest.

- Path analysis demonstrated that ear prolificacy and plant height had significant (P<0.05) direct effects on grain yield.

- Positive and negative significant (P<0.05) indirect effects on grain yield were observed on; ear prolificacy via silking days and plant height, and plant height via silking days, stalk lodging, ear prolificacy and grain moisture content at harvest.

9.3.3 Assessment of genetic gains: I. Performance *per se*

A total of 76 introgressed lines generated from introgression of temperate germplasm into tropical elite maize inbred lines were evaluated for genetic gains and performance *per se* improvement for grain yield and its components relative to tropical and temperate parental inbred lines as negative and positive controls, respectively.

- Positive realised genetic gains were achieved for grain yield (5%) and ear prolificacy (46%) relative to the mean of checks.

- Plant aspects such as plant and ear height, root and stalk lodging had gains ranging from 2% to 11%.
- Plant attributes for early physiological maturity; anthesis and silking days, and grain moisture content at harvest had low gains.
- Introgressed lines performance *per se* showed improved performance for plant and ear height, and grain yield potential.
- Introgressed line 71-DMLF7-88 had positive improvement relative to the mean of checks combining early physiological maturity, high ear prolificacy and grain yield potential.

9.3.4 Assessment of genetic gains: II. Performance *inter se*

Single cross hybrids generated from test crossing introgressed lines to tropical elite inbred line testers were evaluated for genetic gains, and performance *inter se* improvement for grain yield and its components relative to commercial check hybrids.

- Grain yield and ear prolificacy had positive realised genetic gains of up to 58% and 26%, respectively, relative to the population mean and commercial check hybrids.
- Secondary traits such as anthesis and silking days had gains ranging from 1% to 37%.
- Negligible gains were achieved for stalk and root lodging, and grain moisture content at harvest.
- Introgressed lines performance *inter se* indicated significant improved performance for grain yield and its components.
- Single cross hybrids; 12C20264, 12C22766, 13XH349 and 11C1774 combined high yield potential, low grain moisture content at harvest and improved standing ability relative to the commercial check hybrids.

9.3.5 Stability and adaptability of hybrids
- Assessment of yield stability and genotype adaptability of the single cross hybrids across the South African warm temperate environments relative to adapted commercial hybrid checks.

- GGE-biplot analysis revealed two mega environments in the hybrid maize hybrid trials
- Ukulinga Research Station had the ability to clearly discriminate hybrids according to yield potential
- Six winning genotypes namely; 25 (12C19813), 89 (12C20628), 108 (13XH344), 110 (13XH346), 112 (13XH349) and 128 (13XH1060) were identified for South African warm temperate environments.

9.3.6 Economic appraisal of alternative breeding strategies

In this study, a cost benefit analysis of conventional and "shuttle breeding" programmes in breeding for adaptability to South African warm temperate environments was carried out. Cost benefit analysis was based on spreadsheet budgets and revenue cash flows adjusted for time value-net present value (NPV).

- The conventional breeding programme carried out in Zimbabwe had a high NPV ($1, 779, 084 .00) (13%) relative to conventional breeding programme carried out in South Africa.
- Positive NPV of $1, 834, 166 .00 was achieved for the "shuttle breeding" programme. This indicated a 17% and 3% higher NPV in comparison with conventional breeding programmes carried out in South Africa and Zimbabwe, respectively.
- In the long term, the "shuttle breeding" programme had the highest NPV, and the advantage of increasing shareholder value relative to both conventional breeding programmes.

9.4   Implications of the findings for breeding

The study on molecular characterization of introgressed lines bred in different environments using 20 SSR markers identified genetic diversity among introgressed lines developed in the same environment. This illustrates that breeding programmes may exploit the random effects of selection environments during breeding to ensure establishment and maintainance of genetic diversity in a population. Accurate assessment of this genetic diversity guarantees selection of appropriate genetic base

populations of elite germplasm and generation of progenies with lowest similarity for further selection. Most importantly in this study, introgression of temperate germplasm into tropical maize elite inbred lines did not disrupt heterotic groupings. This observation suggests that breeders can ensure that the new (introgressed) lines are appropriately selected within their inclined cluster for hybrid combinations to maximise on hybrid vigour. In addition, new inbred lines within clusters can be used for population improvement. The findings of introgressed lines that did not show inclination to original heterotic groups may be attributable to recombination that resulted in recombinant lines that can be exploited in breeding programmes. This infers that breeding programmes are likely to achieve maximum hybrid vigour from hybrid combinations across clusters with the non-aligned clusters.

Evaluation of introgressed maize inbred lines for selected economic traits and grain yield revealed significant genetic variation among the introgressed lines. Heritability estimates ranged from low (21%) to high (93%). This suggests that the selected traits and grain yield were under genetic control which could result in effective selection during trait improvement. Bello et al. (2012) reported that the amount of genetic variation and level of heritability determined the rate of breeding progress during crop improvement. This would imply, therefore that breeding programmes can explore this heritable genetic variation to improve desired economic traits. Introgressed lines selected based on ear prolificacy and grain yield potential across sets illustrated effectiveness of introgression strategy in improving performance. Grain yield potential of selected introgressed lines indicated environmental effect on selection of introgressed lines. This observation warrants the need to consider the effects of genotype-by-environment to ensure effective selection

Some selection environments were shown to be positively correlated hence the need to use only one of the corresponding environments during selection. Correlation among traits indicated that grain yield had significant correlations with secondary traits such as; plant and ear height, root and stalk lodging; and grain yield components; ear prolificacy and grain moisture content at harvest. Path analysis further demonstrated that correlations observed for ear prolificacy and plant height

had moderate direct effects on grain yield. This implies that these traits should be given a high preference during selection for high yield in introgressed lines. Significant negligible indirect effects on grain yield were observed for ear prolificacy via silking days and plant heights; and plant height via silking days, stalk lodging, ear prolificacy and grain moisture content at harvest. This suggests the need to give these traits low selection preference during breeding.

Results of introgressed lines' genetic gains and performance *per se* improvement achieved through introgression of temperate germplasm into tropical elite inbred lines demonstrated positive realised genetic gains for grain yield and its components. Gains that were achieved for grain yield, ear prolificacy, plant and ear height, and root and stalk lodging showed that the introgression strategy was effective in improving traits that are important for the South African warm temperate environments. This observation highlighted that the introgression breeding strategy can effectively be used in enhancing adaptability of tropical germplasm to the South African warm temperate environments. A number of introgressed lines showed improved performance *per se* across traits, with introgressed line 71-DMLF7_88 illustrating outstanding performance *per se* that combined early physiological maturity, high ear prolificacy and comparable grain yield relative to the mean of temperate checks.

Positive genetic gains and performance *inter se* improvement of the introgressed lines was realised for grain yield and ear prolificacy with low to negligible gains for the secondary traits. This was an indication that introgression of temperate germplasm into tropical elite maize inbred lines was effective in improving traits (grain yield, ear prolificacy, anthesis and silking days) that are important in South African warm temperate environments. This observation denotes that introgressing of temperate germplasm into tropical elite inbred lines is an effective tool for enhancing adaptability in temperate environments. Introgressed lines *inter se* performance identified outstanding hybrids (12C20264, 12C22766, 13XH349 and 11C1774) that combine desired traits. This may suggest that introgressed lines involved in the hybrid combinations had improved adaptability for South African warm temperate

environments. Therefore, these inbred lines can be advanced in the programme and form the basis of future projects for adaptation to temperate environments.

Stability and adaptability assessment of single cross hybrids using GGE-biplot analysis revealed that there were two mega environments identified in this study. This infers that breeding programmes should be able to define their target environments to ensure effective testing during multi-locational trials. Introgression of temperate germplasm in tropical germplasm was effective in improving grain yield potential as indicated by six winning experimental single cross hybrids 25 (12C19813) 89 (12C20628), 108 (13XH344), 110 (13XH346), 112 (13XH349) and 128 (13XH1060) relative to the adapted hybrid checks. These hybrids can be recommended for advanced testing in multi-locational trials. This observation entails that breeding programme should be able to identify high yield and adaptable maize hybrid varieties they can recommend in target environment for farmer uptake.

Economic appraisal of the conventional and "shuttle breeding" programmes for breeding and subsequent commercialisation of tropical germplasm for adaptability to South African warm temperate environments showed that the "shuttle breeding" programme had the highest net present value. This observation infers that small and medium scale seed companies' programmes operating outside South African warm temperate environments can cost effectively breed for this market using a "shuttle breeding" programme. However, it should be noted that this is a long term project in which recoup of initial investment costs takes longer than use of conventional breeding programmes. Genetic gains attained through the use of the "shuttle breeding" programme indicate that there is need for a trade-off between increasing shareholder value and a long–term sustainable programme that releases competitive maize hybrid varieties. The genetic gain is likely to be greater when crop varieties are bred in the environment of potential deployment. Breeding programmes intending to utilise the "shuttle breeding" programme should identify appropriate base populations; and only the F_3 families and their test crosses should be evaluated in South Africa. In addition, multi-locational hybrid trial evaluations can be restricted to a few sites that are a true representation of target environments. This will result in further increase in

NPV thus meeting the corporate objective of increasing shareholder value and releasing productive maize hybrid varieties. However, it should be noted that the economic climate in both environments can change over time hence the need to continuously monitor economic trends to ensure relevance of the breeding programmes.

9.5   Conclusions

The aim of the study was to assess the effectiveness of introgression of temperate germplasm into tropical elite maize inbred lines as a strategy to enhance adaptability of new hybrids to South Africa warm temperate environments. To this end, the following conclusions could be drawn.

9.5.1 Effects of introgression on genetic diversity and heterotic patterns

Introgression of temperate germplasm into tropical elite inbred lines did not disrupt the heterotic groupings because most of the introgressed lines (86%) fitted into known existing heterotic groups. Only 14% of the introgressed lines did not show any inclination towards the known heterotic sub clusters of their founder tropical parents. These lines were considered to be new recombinant inbred lines that showed little resemblance with their founder parents. Selection environment did not influence heterotic clustering of the introgressed lines. However, genetic diversity was identified among introgressed lines developed in the same environment, and also between environments.

9.5.2 Genetic variation and relationships

Genetic variation was observed for the major economic traits and heritability estimates ranged from low (21%) to high (91%). Comparison of means of inbred line sets showed the introgression was effective for improving grain yield potential and ear prolificacy of the tropical elite inbred lines. Spearman's rank correlation analysis on grain yield and ear prolificacy data showed significant positive correlation between

selection environments such as Ukulinga in South Africa and Kadoma Research Centre in Zimbabwe. Therefore Kadoma Research Centre will be recommended for use in breeding new maize germplasm lines for South Africa. Correlation among traits showed that ear prolificacy and plant height had significant (P<0.05) direct effects on grain yield thus these traits will be emphasised in breeding new hybrids for South African warm temperate environments. The secondary traits had negligible indirect effect on grain yield indicating that indirect selection of these traits would not influence grain yield potential of hybrids.

9.5.3 Genetic gains and performance of introgressed lines

Introgression of temperate germplasm into tropical elite maize inbred lines was effective for improving their adaptation to South African warm temperate environments. Positive genetic gains were realised for grain yield (5-58%) and ear prolificacy (26-46%) after just one breeding cycle. Further secondary traits such as plant and ear height, anthesis and silking days had gains ranging from 1% to 37%. However, economic traits such as root and stalk lodging, and grain moisture content had low gains achieved, indicating that a lot of work is still to be done to improve the agronomics of the hybrids. However, introgressed lines performance *per se* and *inter se* was impressive as new lines with potential for commercial production were identified. Inbred line 71-DMLF7_88 combined early physiological maturity, high ear prolificacy and high yield potential qualifying it as a perfect parent for use in productive hybrids for the warm temperate environments in South Africa. Exceptional hybrids like 12C20264, 12C22766, 13XH349 and 11C11774 were also identified for advancement in the programme that emphasise high productivity in warm temperate environments.

9.5.4 Stability and adaptability of hybrids

The study was also successful at identifying hybrids that combined high productivity and stability at the level of standard commercial hybrids. The six hybrids: 25-12C19813, 89-12C20628, 108-13XH344, 110-13XH346, 112-13XH349, and 128-13XH1060 were identified as stable and adaptable relative to standard commercial hybrid checks. Four hybrids, namely, 75-12C20595, 112-13XH349, 225-12C22785, and 246-11C1774 were considered as ideal alongside the commercial hybrid check PAN6Q445B giving credence to achievement of significant gains in yield, because PAN6Q445B is a market leader in South Africa's warm temperate environments.

9.5.5 Economic gain

The study also indicated significant economic gains when a "shuttle breeding" programme is implemented to breed new hybrids following the introgression strategy. The "Shuttle breeding" programme attained a positive net present value (NPV) of $1, 834, 166. 00. This indicated an increase in shareholder value through an opportunity cost of 17% and 3% relative to conventional breeding programmes which are based in South Africa and Zimbabwe, respectively. Positive NPV and genetic gain achieved using the "shuttle breeding" programme makes it a viable option for small and medium scale seed companies intending to breed and commercialise competitive products in South African temperate environments.

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