

**Genetic Variation and Associations among Adaptive Traits in a Recombinant
Maize Inbred Line Population**

By

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General abstract

Maize production in Africa is constrained by abiotic and biotic stresses. Breeders need to have information on the nature of combining ability of parents, their traits and performance in hybrid combination. This requires careful determination of genetic variability of parents, and studying associations between grain yield and adaptive traits to breed superior cultivars which are better able to withstand such stresses. Therefore, this study was aimed at selecting parental testers with best combining ability in hybrid combination with recombinant inbred lines (RILs); and studying the correlation between grain yield and its components in eastern and western South Africa. It was also aimed at determining genetic variation and associations among adaptive traits in hybrids involving RILs. The final objectives of the study were to determine cultivar superiority of testcrosses involving RILs, and to select the best cultivars within and across four different environments.

The 42 RILs were crossed to 9 Zimbabwean tropical testers resulting in 1009 hybrids with sufficient seed for planting in trials. From these a sample of 87 hybrids with adequate seed were selected and planted at four sites for combining ability analysis. The hybrids were evaluated at four sites in two regions; western region (Potchefstroom research station) and eastern region (Cedara, Ukulinga and Dundee research stations), during 2011/12 season. The experiments were laid out as augmented alpha lattice design. Trials were managed in accordance with production culture for each region. All quantitative data was subjected to GenStat and SAS statistical softwares.

The results from combining ability study indicated that the line general combining ability (GCA) effects played a non-significant role ($p > 0.05$) in determining grain yield, grain moisture and anthesis date, while they were significant ($p \leq 0.05$) for the other traits such as ear prolificacy. The tester main effects were significant for all the traits except ear prolificacy and plant height. Results also revealed that all the traits were controlled by both additive and non-additive genes, where additive gene action had the most contribution to the traits. The non-additive gene action played a minor role suggesting the total GCA effects attributed to both lines and testers predominantly higher over the specific combining ability (SCA) for all traits. In general the additive effects were preponderant over the non-additive

gene effects. One cross (L114 x T12) had a significant and positive SCA effect for grain yield. The correlation between grain yield and secondary traits (number of ears per plant, grain moisture content, ear height, plant height, ear position and anthesis date) suggested that indirect selection can be employed to enhance grain yield by breeding for these particular adaptive traits. Path analysis showed that plant height had the highest direct and indirect effect on grain yield indicating its importance among other secondary traits for grain yield enhancement. Phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the studied traits across all the four environments. All the traits displayed high heritability at Potchefstroom except anthesis date which was highly heritable at Ukulinga. Cedara was the second best site for heritability of all the traits except for the number of ears per plant. The genetic advance for grain yield was the highest at Cedara followed by Potchefstroom, Dundee and Ukulinga. The hybrids exhibited different patterns of variation and distribution for all the traits. This indicated that selection strategies to exploit GCA should be emphasised.

Association studies among grain yield and secondary traits such as ear length, number of ears per plant, plant height, anthesis date, silking date and ear leaf area revealed that there were significant phenotypic correlations between grain yield and secondary traits, and among the secondary traits. Ear length had the highest direct effect on grain yield at Ukulinga; number of ears per plant had the highest direct effect on grain yield at Cedara and Potchefstroom; whereas plant height had the highest direct effect on grain yield at Dundee. Grain yield was least affected by indirect factors at all the sites except Ukulinga, where anthesis date had the highest indirect effect on grain yield through silking date followed by plant height through leaf area. The study reveals that there is significant variation among the hybrids for mean performance, indicating that there is opportunity for selection. Overall the findings suggest that direct selection would be appropriate to enhance grain yield. Path analysis revealed that plant height had the highest direct and indirect effects on grain yield, indicating that plant height can be further exploited as the main trait in future breeding programmes for grain yield increment.

Hybrid 10MAK10-1/N3 was the best hybrid at Ukulinga in terms of grain yield, relative yield and economic traits. Whereas hybrid T17/L83 was the best hybrid at Cedara in terms of grain yield and relative yield; however, T11/L102 was selected as the most elite hybrid with respect to grain yield, relative yield and economic traits. Hybrid T3/L48 was identified as the best hybrid at Dundee with respect to grain yield, relative yield and prolificacy. At Potchefstroom the standard check PAN6611 was identified as the best hybrid in terms of grain yield and relative yield followed by developmental hybrid T1/L28; however, developmental hybrid T1/L28 was the best in terms of earliness, prolificacy and ear aspect. Stability coefficients and cultivar superiority index across the sites revealed that four developmental hybrids were identified as best hybrids and they performed better than the standard check. These hybrids will be recommended for further testing in advanced trials.

With respect to cultivar superiority, the desired hybrids are required to combine high grain yield with economic and adaptive traits such as high ear prolificacy, low grain moisture, and low ear aspect score (desired) for them to adapt to production environments in South Africa. There was significant variation among the top 25 yielding hybrids. At least 5 hybrids combined high grain yield with the desired complimentary adaptive traits such as quick moisture dry down, prolificacy and ear aspect. The results showed that there is variation in the performance of high yielding genotypes within all the sites, and that agronomically superior cultivars can be identified.

The study shows that there is significant variation among the RILs since they interacted differently with the 9 tropical testers. Even among the top 25 selections of RILs in each environment there was still variation for combinations of the desired traits. Significant associations among grain yield and other economic and adaptive traits were observed with implications for breeding strategy. Above all the significant variation gives large score for future breeding of new unique lines.

Declaration

I, Mxolisi Percival Sibongeleni Sithole, declare that:

1. The research reported in this dissertation, except where otherwise indicated, is my original research.
2. This dissertation has not been submitted for any degree or examination at any other university.
3. This dissertation does not contain other person's data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
 - a. Their words have been re-written but the general information attributed to them has been referenced.
 - b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.
5. This dissertation does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the references sections.

Signed

.....

Mxolisi Percival Sibongeleni Sithole

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

.....

Prof. John Derera (Supervisor)

.....

Dr. Alfred Odour Odindo (Co-Supervisor)

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Dedication

To *God Almighty* for being *My All*. This piece of work is dedicated to my late great grandmother, MaKhumalo. She is the structural component of everything that I am today.

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List of Abbreviations

cm = centimetre

cm² = centimetre squared

cm⁻² = per centimetre squared

CV = coefficient of variation

DF = degrees of freedom

FAO = Food and Agriculture Organisation

ha = hectare

H² = broad sense heritability

IITA = International Institute of Tropical Agriculture

kg = kilogram

kg/ha = kilogram per hectare

m = metres

mm = millimetre

MSE = mean square error

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

N = North

NDA = National Department of Agriculture

no. = number

QTL = Quantitative Trait Loci

S = South

SE = standard error

t = tonnes

t ha⁻¹ = tonnes per hectare

t/ha = tonnes per hectare

V_G = genetic variance

°C = degree celsius

% = percentage

%SS = percentage sum of squares

INTRODUCTION TO DISSERTATION

Importance of maize

Maize (*Zea mays* L., *Poaceae* family) is one of the most important food crops in the world. Together with other important staples such as rice and wheat, maize provides at least 30% of the food calories to more than 4.5 billion people in 94 developing countries (Shiferaw et al., 2011). Maize grows widely throughout the world in a range of agroecological environments (IITA, 2009). In addition to food and feed, maize has wide range of industrial applications such as in food processing to manufacturing of ethanol (Abbassian, 2006). Also, maize accounts for 30-50% of low-income household expenditures in Eastern and Southern Africa (UNDP, 2010). Thus, the scarcity of maize is undoubtedly accompanied by negative effects on food and feed markets (Anonymous, 2009). It is also the principal food and feed crop of South Africa; hence it impacts on food security.

According to van Biljon (2010), maize yields have changed over the last 25 years, its average yield for South Africa increased from approximately 2.3 tons ha⁻¹ in the early 1980s to approximately 3.5 tons ha⁻¹ in recent years. Maize is the most important grain crop in South Africa, being both the major feed grain and the staple food for the majority of the South African population (NDA, 2011). About 60% of maize produced in South Africa is white kernel maize and the other 40% is yellow maize (NDA, 2011). Most of the white maize is consumed directly as food with small quantities as other uses (FAO, 1997); while most of the yellow maize is used for feed and industrial processing. Maize is produced throughout South Africa with Free State, Mpumalanga and North West provinces being the largest producers, accounting for approximately 84% of total production, and is produced mostly on dry land although there is less than 10% that is produced under irrigation (NDA, 2011).

Adaptation ability of maize

Maize is a tropical crop that is well adapted to many climates and hence has wide-ranging maturities from 70 days to 210 days (Belfield and Brown, 2008). Maize is produced throughout South Africa under diverse environments (du Plessis, 2003). Maize needs 450 to 600 mm of water per season, which is mainly acquired from the soil moisture reserves (du Plessis, 2003). No other crop utilises sunlight more effectively than maize, and its yield per

ha is the highest of all grain crops (du Plessis, 2003). The optimum temperature for maize growth and development is 18 to 32°C, with temperatures of 35°C and above considered inhibitory (Belfield and Brown, 2008), and this leads to the decline in yield production and poor crop performance unless the crop is adapted to such conditions. In South Africa, maize is produced in 5 production regions, namely western, temperate eastern, cold eastern, KZN region and irrigation/cold to temperate region. In the current study, maize trial experiments were conducted in the western and KZN regions which represent the major production domains, namely the western and eastern maize belts.

Maize production constraints

There is a need to develop stress tolerant maize hybrids due to their increasing demand as a result of challenges posed by increasing climate change, and increasing water, nutrient and land costs (Bodnar, 2010). Increasing demands and decline in global maize supplies have weakened market volatility and somehow resulted to increased global maize prices (Shiferaw et al., 2011). Climatic variability and change, and the consequent rise in abiotic and biotic stresses, further exacerbate the problem (Shiferaw et al., 2011). Hence, there are a number of factors which limit maize production. The developed countries employ intensive inputs and highly mechanized monocrop production systems using hybrid maize varieties, but in sub-Saharan Africa, majority of maize produced hails from small-scale production and is generally used for subsistence in a multiple cropping system, intercropping and mixed farming systems, because animal and crop production are combined (M'mboyi et al., 2010). In most subsistence farming systems of Africa there is a lack of inputs such as fertilizer, improved seed and irrigation (M'mboyi et al., 2010) which calls for a different strategy for breeding appropriate hybrids for low inputs agro-ecologies. A combination of uncertain and variable rainfall, poor soil fertility, high insect pests and disease pressures and lack of well-established marketing systems and infrastructure hamper productivity of maize (M'mboyi et al., 2010).

The bio-physical constraints in maize production include biotic and abiotic factors. The biotic constraints such as diseases, pests, and weeds (Ekasingh et al., 2004) are very prevalent in tropical environments due to high temperature and humidity conditions. The abiotic

stresses are drought and low and declining soil fertility (Ekasingh et al., 2004). Due to limitations of infrastructure most maize production in Africa is rainfed ($\geq 90\%$) (IITA, 2009), hence erratic rainfall has serious consequences for food security and poverty in predominantly agro based economy. In developing countries, production is largely dependent on climatic conditions which can only be partially manipulated by man through irrigation (NDA, 2011). Unfortunately only 10% of the maize is irrigated in South Africa. Thus, this calls for the development of drought tolerant and generally adaptable maize for South Africa, and in other similar environments elsewhere.

Justification of the current study

Productivity of maize hybrids is compromised by biotic and abiotic stresses such as drought among other factors. However, stress tolerance can be enhanced by improving the adaptive traits in hybrids. The following adaptive traits were evaluated in this study; grain yield, number of ears per plant, grain moisture content, ear length, ear height, plant height, ear position, plant density, anthesis date, silking date, anthesis-silking interval, number of tassel branches, kernel rows per ear, number of kernels per row, number of kernels per ear, number of leaves, chlorophyll content, ear leaf area, disease reaction to grey leaf spot (GLS) and phaeosphaeria leaf spot (PLS), and ear aspect. Prior to selection for adaptive traits there is a need to determine genetic variation for these traits in the breeding base population. There is a need also to quantify the diversity of the base inbred population. The information to be generated will be used to devise an appropriate breeding strategy that aims to achieve hybrids with adaptation ability under production conditions in South Africa.

According to Moreno et al. (2005) drought and low-temperatures among other factors, negatively affect plant growth resulting in devastating yield reductions worldwide. Hybrids which are stress tolerant and highly adaptable to environmental challenges are required. Unfortunately drought-tolerance traits are not always associated with a better grain yield (Moreno et al., 2005). Therefore, it is of utmost importance to determine the relationship between these adaptive traits and overall yield as a basis for devising the breeding strategy for South Africa. In western South Africa, drought is prevalent while temperatures go down quickly towards end of the season as winter approaches. Therefore, appropriate hybrids

design should combine grain yield with the following traits: low grain moisture (earliness), prolificacy and lower ear aspect score (desired).

Objectives of the study

The main objective of the study was to determine genetic variation for adaptive traits and establish the associations between these traits and grain yield in a recombinant inbred line (RIL) population. The study of the RILs was mainly done via their hybrids because the breeding programme emphasises hybrids. It is long established that there is no strong correlation between inbred line performances per se with the hybrids.

The specific objectives of the study were to:

- a. Determine combining ability of recombinant maize inbred lines with tropical testers;
- b. Determine genetic variation and associations among adaptive traits in hybrids involving maize recombinant inbred lines;
- c. Determine cultivar superiority of testcrosses among recombinant inbred lines

Research hypotheses

The following hypotheses were formulated:

- a. There is large genetic variation for adaptive traits which can be exploited to breed new hybrids;
- b. Adaptive traits are controlled by additive gene effects, therefore are highly heritable;
- c. There is a significant correlation between adaptive traits and productivity in the RILs and knowledge of this relationship can be used to pinpoint the best selection strategy for use in the programme;
- d. The RILs are genetically divergent from the standard testers in the breeding program; therefore they would combine well with the testers with implication for breeding superior cultivars.

Structure of the dissertation

This dissertation is made up of six main sections that include six chapters as shown below:

Chapter 1: Literature review

- Chapter 2: Characterisation of recombinant maize inbred lines
- Chapter 3: Combining ability of recombinant maize inbred lines with tropical testers
- Chapter 4: Genetic variation and associations among adaptive traits in maize recombinant inbred lines
- Chapter 5: Cultivar superiority of testcrosses among recombinant inbred lines
- Chapter 6: General overview of the study and future directions.

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Chapter 1: LITERATURE REVIEW

1.1 INTRODUCTION

This chapter reviews the literature on the development of stress tolerant and high yielding maize varieties. It starts by introducing the crop, followed by reviews on stress and maize production with particular attention on drought stress and potential losses that can be incurred due to drought stress. The effect of drought stress at different growth stages in the maize growth cycle, and the mechanism of drought tolerance also form part of the review. Also, important sections include: adaptive traits, genetic variation, heritability, relationship between adaptive traits and yield, path-coefficient analysis, combining ability with emphasis on line by tester analysis, and testers with emphasis on their use and importance. Conclusions drawn from the review are provided at the end of the chapter.

1.2 MAIZE

Maize (*Zea mays* L.) originated in Mexico (Mangelsdorf et al., 1964; as cited by M'mboyi et al., 2010). Its origin dates back to at least 7000 years in the form of *teosinte* in Central Mexico (Abbassian, 2006). The spread of maize was facilitated by trade and establishment of colonies (Burt-Davy, 1914). There is a lot of diversity in maize which is also reflected through grain texture such as dent or flint, and sweet or green maize (Anderson and Cutler, 1942). Depending on their colour and taste, maize grown around the world is generally categorized into two broad groups such as yellow or white (Abbassian, 2006). White maize is the main focus of this study. It is generally considered as a food security crop in Africa (Abbassian, 2006).

1.3 STRESS AND MAIZE PRODUCTION

Stress is a condition in which increasing demands made upon a plant lead to an initial destabilization of functions, followed by normalization and improved resistance (Larcher, 1987; as cited by Bänziger et al., 2006). If the limits of stress tolerance are exceeded and the adaptive capacity is surpassed, permanent damage or even death may result (Bänziger et al., 2006). This results in the ultimate loss of yield. Drought and nitrogen (N) stress are the two most important factors limiting maize production, especially in developing countries

(Edmeades et al., 1989; as cited by Bänziger et al., 2006). Maize is the most drought susceptible of all cereals (Bodnar, 2010). Combining knowledge of yield sustaining traits under drought and introgression of the most effective Quantitative Trait Loci (QTLs) into elite hybrids without harming yield of the recipient can enhance potential yield in maize (Cattivelli et al., 2008). Thus, introgression of desired QTLs can be used to reduce the gap between yield potential and actual yield under stress (Cattivelli et al., 2008). Enhancing yield of hybrids under stress can go a long way in alleviating poverty and improving food security. Drought and low soil fertility are among the most important stresses threatening maize production, food security and economic growth in southern and eastern Africa (Bänziger and Diallo, 2001).

1.4 EFFECT OF STRESS ON YIELD AND ITS COMPONENTS

Stress especially drought can result in plants ranging from barren plants with no ears or improved starch content to varying levels of starch in grain depending upon the levels of stress at pollination and subsequent kernel abortion (Mahanna et al., 2012). Stress can result in stand loss, incomplete kernel set, decreased kernel weight, yield loss, and premature plant death. Stand loss is very detrimental during grain fill compared to the vegetative stage and can result in greater yield loss (Nielsen, 2011). When stand loss occurs prior to pollination, ear size on surviving plants may compensate in response to the lesser competition of a thinner stand (Nielsen, 2011). Thus, stronger stems are least susceptible to stand loss. Yield loss as a result of stand loss may be attributed to several factors including poor stem development when plants are exposed to stress.

Kernel set refers to the degree to which kernels have developed on the cob (Nielsen, 2011). Drought at flowering affects silk emergence and captivity, and reduces pollen vigour, resulting in limited kernel set and low yield (Rupitak et al., 2011). Pollen may also die as a result of extreme temperatures. Lower kernel weight and size result in lower yield. Drought from 4 weeks to 66 days after plant emergence will reduce ear size and potential yield (Heiniger, 2001). Yield losses will be related to the length and severity of drought (Heiniger, 2001). It is reported that once grain has reached physiological maturity, stress will have no further negative effect on final grain physiology (such as grain size and texture) and yield.

The amount of yield loss that occurs during dry weather depends on the growth stage of maize and severity of the stress (Heiniger, 2001). Potential maize yield losses due to drought during emergence to eighth leaf growth stage could be as high as 20%; at eighth to sixteenth leaf growth stage could range from 10 to 30%; around flowering and pollination could be 3 to 8% for each day of stress; silking stage to maturity 2.5 to 5.8% with each day of stress (Rafiee et al., 2011). Thus, maize yield is most sensitive to water stress during flowering and pollination, followed by grain filling and finally vegetative growth stage (Rafiee et al., 2011).

Premature death of leaves results in yield losses because the photosynthetic “factory” output is greatly reduced (Nielsen, 2011). Water availability is one of the most important factors in photosynthesis, and its absence or scarcity may consequently result in premature plant death depending on the duration of exposure to such conditions and degree of plant water stress resistance or tolerance. Approximate yield losses due to premature whole plant death range from 12 to 50% when the whole plant death occurs at half-milkline, full dough and dent stages of kernel development, respectively (Afuakwa and Crookston, 1984).

1.5 EFFECT OF DROUGHT STRESS TIME ON MAIZE GROWING CYCLE

1.5.1 Effect of pre-anthesis drought

Pre-anthesis drought significantly reduces the number of kernel rows, the number of kernels per row, as well as the kernel weight (Moser et al., 2006). The adverse effects of pre-anthesis drought on grain yield can be mitigated if varieties are selected for roots which rapidly penetrate the soil and exploit the water resources in deep soil layers (Moser et al., 2006).

Water stress significantly reduces germination percent, germination rate, root length, shoot length, seedling length and seed vigour (Khodarahmpour, 2011). It is necessary to identify hybrids tolerant to drought at the primary growth stage (Khodarahmpour, 2011). If water is limited during vegetative growth, the final leaf area will be smaller and, thus, carbon gain will be reduced throughout the growing season (Nilson and Orcutt, 1996) which will subsequently result in loss of grain yield. In addition, the process of storage reserves in the

stem and ear shank is affected mainly by the conditions under which assimilation takes place before flowering (Nilson and Orcutt, 1996). Prolonged drought stress during the vegetative stages affects the length of internodes by influencing the cell size development and, thus, the capacity for storing assimilates is dramatically reduced (Denmead and Shaw, 1960; Moser, 2004). Under favourable conditions, reserves contribute little to reproductive success (Schussler and Westgate, 1995). However, when photosynthesis is limited during grain filling, the remobilization of stem reserves is considered to be the main source of carbohydrates during grain filling (Blum, 1996), which can sometimes result in weaker stems if the extent of remobilization is greater. The immediate impact of water deficit on the effective leaf area (smaller leaf area as a result of leaf rolling) largely determines the extent of assimilation under drought (Blum, 1996). Plasticity in leaf area development is an important strategy of a drought-stressed crop for maintaining control over water use (Blum, 1996). In drought-sensitive landraces, water deficiency significantly decrease the number of leaves, root volume, total leaf area and plant dry weight (Fang et al., 2011). Water stress has little effect on both morphology and physiology of the drought-tolerant landraces (Fang et al., 2011). This suggests that some genotypes can confer resistance when exposed to pre-anthesis drought. Differences in tolerance to water stress exist among different types of maize landraces, and suggest that biomass and nitratase could be regarded as their screening indexes for traits tolerant to water stress at seedling stage (Fang et al., 2011). Plant breeders are interested in the variation among genotypes upon which selection of adapted hybrids is applied.

1.5.2 Effect of drought stress during reproductive stage

Maize is especially sensitive to drought at flowering (Grant et al., 1989). Abortion of ovules, kernels, and ears occurs from one week before silking to two weeks after silking (Moser, 2004) which is accentuated by drought and heat stress occurring at these critical stages (Uhart and Andrade, 1995). From various studies, it is suggested that water and/or N deficiency reduce carbon availability and dry matter partitioning to the ear during the critical period that determines grain number (Uhart and Andrade, 1995). It is generally accepted that, when drought begins to affect the plant during reproduction, the plant decreases the reproductive demand for carbon by reducing the number or size of the sinks

(Moser, 2004). Consequently, tillers may degenerate, flowers drop, pollen die, and ovules abort (Blum, 1996). Edmeades et al. (1993) also reported that when the supply of assimilates to the ear falls below the threshold necessary for ovules to develop, all the kernels abort, resulting in a barren plant. Low water potential during anthesis does not delay pollination, but prevents the development of embryos due to a lack of photosynthates (Westgate and Boyer, 1986).

1.6 BREEDING FOR DROUGHT STRESS TOLERANCE

Drought tolerant crops are those which are better able to withstand limited water supply; they are expected to perform better than “regular” maize under moderate drought by 25 to 30%, which results in higher yield (FarmingFirst, 2009) compared to the susceptible varieties. Progress in breeding for stress tolerance in maize has been reported. Badu-Apraku and Akinwale (2011) identified superior inbred lines for use as parents for hybrids production and for introgression into maize breeding populations. Ten inbreds were identified as the most promising parents under drought stress (Badu-Apraku and Akinwale, 2011). Four inbreds combined tolerance to drought stress and low N and could be used as germplasm sources for introgression of tolerance genes in hybrids to enhance adaptation (Badu-Apraku and Akinwale, 2011). Under drought stress, four inbreds were the closest to the ideal genotype, while the other four inbreds were the closest under low-N conditions (Badu-Apraku and Akinwale, 2011). Extra-early inbreds and hybrids are not only drought escaping but also possess drought and low-N tolerant genes (Badu-Apraku et al., 2011).

Increased leaf longevity, increased water and nutrient uptake, greater assimilate supply during grain filling, and increased grain and ear set have been associated with constitutive stress tolerance mechanisms in maize (Bänziger et al., 2002). Maize with adaptive changes associated with drought tolerance that are sustained under N stress may indicate constitutive stress tolerance mechanisms (Bänziger et al., 2002). Decreased ear abortion and increased assimilate supply during grain filling of maize selected for tolerance to mid-season drought also provide tolerance to N stress and therefore may contribute to increased yield and yield stability (Bänziger et al., 2002). Selection for these traits can be used to improve stress tolerance.

Irrespective of the timing of drought, the high osmotic adjustment crops have been reported to extract significantly more water from deeper in the soil profile during drought stress period and they exhibited higher leaf area duration and attained greater grain yields, when they were droughted at flowering they exhibited greater harvest index than the low osmotic adjustment crops (Chimenti et al., 2006). Osmotic adjustment can contribute to drought tolerance in maize crops exposed to water deficit both before and during flowering, and that the trait carries no yield penalty under irrigation (Chimenti et al., 2006). This is a good trait to include in the selection index for maize hybrids. On the other hand, Homayoun et al. (2011) reported that stress-resistant genotypes with higher potential yield and chlorophyll content exhibited best performance than none stress-resistant cultivars. Moreover, Izge and Dugje (2011) observed that most entries which gave higher grain yields incidentally produced higher values of grain weight. In the same vein, entries having higher grain yields also flowered earlier and could have an inherent potential for early maturity (Izge and Dugje, 2011).

1.7 GENETIC VARIATION

The understanding of the genetic basis of hybrid performance under stresses is crucial to designing appropriate breeding strategies (Betran et al., 2003). Process of maize breeding to get high yielding hybrids, begins through genetic variability determination of the base population or selected inbred lines (Babic' et al., 2011) which can thus facilitate the selection of the desirable parents. Hence, the genetic divergence of parental inbred lines is a main step to get high heterotic effect in yield after crossing (Babic' et al., 2011). While quantifying the magnitude of genetic variability in breeding maize for improved drought tolerance, Guei and Wassom (1992) reported that additive gene action was more important than dominance in controlling the expression of flowering traits. However, more dominance deviations were detected in yield and prolificacy, and additive genetic variance was larger in magnitude under stress than non-stress, except for yield in one of the populations (Guei and Wassom, 1992). It was concluded that silking date, anthesis-silking interval, and number of ears per plant were correlated with yield in stress environments and may be more effective for screening genotypes in water-stress than non-stress environments. Rafiq et al. (2010) reported substantial variability for all traits studied and that genetic advance was higher for

plant height, ear length, grains per row and grain yield. Knowledge of genetic variation is fundamental in breeding programs, because the plant populations and varieties vary at the genetic level and as a result they have differing phenotypic performance. The knowledge of variability of the breeding material is essential for ease of hybrid development.

1.8 ADAPTIVE TRAITS

Adaptive traits are morphological and physiological characters associated with resistance or tolerance to stress (Chen et al., 1996), which is ultimately reflected by high yield under stress conditions. Plant breeding aims to produce high yielding varieties (Salahuddin et al., 2010), which are adapted to the target environment. There are many factors on which the yield depends which comprise secondary traits such as plant height, number of fruiting branches and seed index (Salahuddin et al., 2010). It is desirable to know the extent of the relationships between yield and its various components or secondary traits. It also happens that due to character association, improvement of one character may have been obtained at the expense of other (Salahuddin et al., 2010). Therefore, it is crucial to find which traits are negatively associated with yield and other traits. Adaptive traits may fall into many groups which can be defined with respect to relevance such as flowering adaptive traits (those that are incurred at flowering), tassel (tassel modification), grain/kernel traits, lodging, leaf modification, ear modification, plant number and height. The knowledge of adaptive traits is essential to know the phenotypic presentation of the genes of interest and is used to develop new varieties, which are adaptable to the target environments.

1.8.1 Role of adaptive traits in selection

The improved performance of drought tolerant population hybrids across environments was due to improvements in secondary traits such as reduced anthesis-silking interval, increased ears per plant, delayed senescence and relatively high leaf chlorophyll during late grain filling (Zaidi et al., 2004). Selection for mid-season drought tolerance resulted in morpho-physiological changes that proved advantageous under both drought and low-N stress, without significant yield penalties under optimal input conditions (Zaidi et al., 2004). Lu et al. (2011) reported that kernel weight was the most stable trait under drought stress. Root capacitance had relatively low heritability and low genetic correlation with other drought

resistance criteria, and is not recommended as a drought resistance criterion (Lu et al., 2011). Some maize lines developed for temperate regions showed strong drought resistance comparable to tropical maize lines when tested under tropical condition, indicating that temperate lines with a wide adaptability can be used in drought resistance breeding for both temperate and tropical environments (Lu et al., 2011). Through heritability of the traits that confer resistance of temperate lines, cross breeding can be performed to transfer resistance into elite tropical lines. This therefore shows how essential the study of heritability is in plant breeding.

Rafiq et al. (2010) reported that grain yield, ear length, ear height and grain weight had high genotypic coefficient of variability (GCV) estimates with high heritability. Heritability is essential in determining the degree of transferability of traits to the progeny, since the level and extent of heritability differs among traits.

1.9 HERITABILITY

Heritability is a quantitative measure which provides information about the proportion of genotypic variance out of the total phenotypic variance (Dabholkar, 1999). The term heritability can be further divided into broad sense and narrow sense, depending on whether it refers to the genotypic or breeding value, respectively (Gebre, 2005). The ratio of genetic variance to phenotypic variance (V_G/V_P) is called heritability in the broad sense or genetic determination (Nyquist, 1991). It expresses the extent to which individual phenotypes are determined by the genotypes (Nyquist, 1991). A large percentage of heritability for a character is regarded as highly heritable whereas if it is smaller, it is regarded as less heritable (Dabholkar, 1999). On the other hand, the ratio of additive variance to phenotypic variance (V_A/V_P) is called heritability in the narrow sense (Gebre, 2005). This expresses the extent to which phenotypes are determined by the genes transmitted additively from the parents to offspring (Lush, 1940 as cited by Pradeepa, 2007). It also expresses the magnitude of genotypic variance in the population, which is mainly responsible for changing the genetic composition of a population through selection (Dabholkar, 1999).

1.10 CORRELATION ANALYSIS

Information on genotypic and phenotypic correlation coefficients among various plant traits helps to ascertain the degree of associations in determining the response to selection (Yousuf and Saleem, 2001). The association between two characters can directly be observed as phenotypic correlation while genotypic correlation expresses the extent to which two traits are genetically associated (Yousuf and Saleem, 2001). Both genotypic and phenotypic correlations among and between pairs of agronomic traits provide scope for applying direct or indirect selection in a breeding programme (Yousuf and Saleem, 2001).

1.10.1 Correlations of yield and yield components

Silking date, anthesis-silking interval, and number of ears per plant correlated well with yield in stress environments (Guei and Wassom, 1992). It has been observed that silking date were positively correlated with ear height and grain yield (Rather et al., 1999). El-Shouny et al. (2005) showed that grain yield per plant correlated positively and significantly with ear length, number of kernels per row, grain weight, number of rows per ear, ear height, plant height and days to silking under normal planting date and with number of kernels per row, grain weight, ear length, number of rows per ear, ear length, number of rows per ear, ear height and days to silking under late planting date. Netaji et al. (2000) reported that yield was significantly and positively correlated with all the characters except anthesis date, silking and dry husk. These results show that there is significant correlation between grain yield, ear components and flowering.

Zhang et al. (2007) reported that the test weight of kernel types was significantly and positively correlated with kernel weight and grain yield. Whereas Li et al. (2006) showed significant positive correlation between kernels per row and kernel yield. Balbinot Jr et al. (2005) also observed that the number of grains per row showed the highest total correlation with grain yield. Prodhan and Rai (2000) also reported that grain yield was strongly associated with grain weight. These studies showed that grain yield has a positive correlation with kernel components. Shelake et al. (2005) found that grain yield was positively and highly correlated with number of grains per cob. While Sofi and Rather (2007) reported that the genotypic correlation coefficient revealed that grain weight, ear length,

number of kernel rows per ear and number of kernels per row showed the greatest correlation with grain yield. Sumathi et al. (2005) reported that ear weight, number of rows per ear, number of kernels per row, and total number of kernels per ear were positively associated with grain yield. Vaezi et al. (2000) observed that grain yield was significantly and positively correlated with ear weight, grain weight and number of kernels per row. Overall, the results show that there are significantly positive correlations between kernel components, ear components and grain yield which show that indirect selections for grain yield can be performed through these traits.

Mohammad et al. (2008) reported that plant height had highly significant association with ear height and anthesis date with silking date. All traits had significant genotypic association but not significant phenotypic association with grain yield (Mohammad et al., 2008). Tan et al. (2006) noticed that grain yield was significantly correlated with plant height, ear length, grain weight and grain production rate. Grain yield was most highly correlated with grain weight, plant height, ear length and grain production rate (Tan et al., 2006). Umakanth and Khan (2001) observed that grain yield showed significant and positive correlations with ear length, plant height and grain weight. Pradeep and Satyanarayana (2001) concluded that grain yield was positively associated with plant height, ear height, ear length, number of kernel rows per ear and grain weight. Kumar and Kumar (2000) suggested that selection based on plant height with greater ear weight, number of kernel rows per ear and number of kernels per ear was desirable for grain yield. Grain yield per plant was positively and significantly correlated with grain weight, number of kernels per ear and ear height (Firoz et al., 1999). Harjinder et al. (2006) reported significantly positive correlations between grain yield, plant height, ear height, and number of ears. These results imply that there is an opportunity for indirect selection for increased grain yield through reduced plant height, early flowering, ear components and kernel components.

It was observed that maximum correlation of grain yield was obtained with number of kernels per row followed by leaf area, plant height, tassel length and ear length (Gautam et al., 1999). Association studies indicated that characters such as plant height, ear height, ear length, number of grains per row, number of grains per ear and grain weight showed

significant positive association with grain yield (Selvaraj and Nagarajan, 2011). While days to tasseling and days to silking showed positive non-significant association with grain yield (Selvaraj and Nagarajan, 2011). Number of rows per ear recorded negative non-significant association with grain yield (Selvaraj and Nagarajan, 2011). Ear length recorded highest correlation with grain yield followed by number of grains per row (Selvaraj and Nagarajan, 2011). Saidaiah et al. (2008) reported a significant and positive correlation between grain yield and plant height, ear height, number of leaves above ear, flag leaf area, chlorophyll content and ear length, except anthesis silking interval and physiological maturity which displayed negative correlation with grain yield. Gholamin and Khayatnezhad (2011) also reported high correlation between chlorophyll content and yield.

The associations between secondary traits and yield also depend on the season. Hefny (2011) reported that under optimal sowing number of rows per ear and number of grains per row exhibited positive and significant correlations at genotypic and phenotypic levels with yield per plant. Anthesis date and silking date associated negatively with grains per row, grain weight and grain yield (Hefny, 2011). Under late sowing conditions, positive and significant genotypic correlations existed between grain yield against anthesis date, silking date, ear length and number of rows per ear (Hefny, 2011).

1.11 PATH-COEFFICIENT ANALYSIS OF YIELD AND YIELD COMPONENTS IN MAIZE

Path-coefficient analysis provides effective means of partitioning correlation coefficients into unidirectional path ways and alternate pathways thus permitting a critical examination of specific factors that produce a critical correlation which can be successfully employed in formulating an effective selection programme in plant breeding (Salahuddin et al., 2010). Path coefficients give the relative contribution of various yield-determining traits, enabling breeders to decide between direct and indirect selection (Makanda et al., 2009b). It is essential to know whether the trait under study has a direct or indirect positive or negative effect on the overall plant yield.

Path analysis revealed that grain weight exerted maximum positive direct effect followed by plant height and number of leaves above ear on grain yield (Saidaiah et al., 2008). Positive indirect effect on yield was by plant height via plant height, ear height, number of leaves

above ear, chlorophyll content, flag area, ear length and grain weight (Saidaiah et al., 2008). Different yield related traits influenced not only through their direct effects but also through indirect contributions towards grain yield (Saidaiah et al., 2008). Singh et al. (2003) observed that ear length had the maximum direct effect on grain yield followed by grain weight and ear leaf area. Number of leaves per plant also had highly positive direct effect on grain yield per plant (Singh et al., 2003). These studies revealed that ear components, grain, height and leaf components could play a significant role when directly and indirectly selected for grain yield improvement and adaptability under stress environments.

Jayakumar et al. (2007) noticed that grains per row recorded maximum positive direct effect on grain yield followed by ear length, days to tasseling and plant height. The maximum negative direct effect on grain yield was recorded by kernel rows followed by days to silking, grain weight, days to maturity, shelling percentage and number of leaves above upper most ear (Jayakumar et al., 2007). These results show that these could be used for indirect selection for grain yield. The number of days to anthesis, number of days to silking and harvest index showed higher genotypic direct effect (Shelake et al., 2005). Kumar et al. (2006) observed that anthesis date, Anthesis-Silking Interval (ASI), ear height and grain weight had the highest direct effect on grain yield. The silking date exhibited negative direct effect on grain yield (Kumar et al., 2006). Therefore, flowering traits can also be indirectly selected for increased grain yield. Arun and Singh (2004) reported that silking date and ear length had the maximum positive direct effect on grain yield. Whereas anthesis date and days to maturity had maximum negative effects on grain yield. Therefore, negative selection for flowering date is essential for increased grain yield.

Path analysis revealed that highest direct effect on grain yield was exhibited by grain weight followed by the number of grains per row, kernel rows per ear and ear length (Rafiq et al., 2010). Most of the traits exerted their positive indirect effects through grain weight, kernel rows per ear and grains per row (Rafiq et al., 2010). It has been revealed that early silking and harvesting of fresh ear, greater plant height, ear length, ear weight, ear height and number of ears per plant directly contributed to increased ear yield (Viola et al., 2003). Bao et al. (2004) reported that maize yield was mainly influenced by ear length, followed by

number of kernels per row, number of rows per ear, growth period and grain weight. These results indicate that ear length is one of the most important factors for grain yield, which thus has implications for direct selection.

The highest positive direct effect on grain yield was exhibited by kernel rows per ear followed by plant height (Singh et al., 1999). The number of kernels per row exerted maximum direct effect on grain yield (Geetha and Jayaraman, 2000; Vaezi et al., 2000). The plant height, silking date, ear length, number of kernel rows per ear, number of kernels per row and grain weight positively influenced the yield directly and also indirectly through several yield components (Swarnalatha and Shaik, 2001). Number of seed rows per ear had a direct positive contribution towards grain yield, ear length, grain weight and number of kernels per row had an indirect negative influence on grain yield (Venugopal et al., 2003). It has been indicated that grain weight had the greatest direct effect on grain yield followed by number of kernels per row, number of kernel rows per ear, and ear length (Sofi and Rather, 2007). Kernel weight per ear mainly affected by ear length, and the ear length with bearing kernel played an important role on grain weight per ear in high yielding combinations (Wang, 2006). These studies reveal that grain components have a highly significant effect on grain yield directly and indirectly which thus have implications for varying breeding strategies.

All traits exerted positive direct effect on grain yield per plant except silking date (Mohammad et al., 2008), indicating importance of earliness in grain yield.

At optimal sowing, ear weight per plant had the highest positive direct influence on grain yield followed by anthesis date and grain weight (Hefny, 2011). On the other hand, silking date exerted high negative direct effect (Hefny, 2011). At late planting, ear weight per plant recorded the highest positive direct effect on grain yield (Hefny, 2011). A moderate and positive influence on flowering traits was observed (Hefny, 2011). Grain number per row recorded negative direct effect on yield and recorded positive and indirect effects through ear weight per plant and grain weight (Hefny, 2011). Therefore, it was concluded that ear weight per plant (at both planting dates), ear length, silking date as a primary; grain weight

and kernels per row as a secondary could be used as the main criteria for yield improvement.

Ear length had a maximum positive direct effect on grain yield followed by ear height, number of rows per ear and days to silking and they contributed primarily to yield and could be relied upon for selection of genotypes to improve genetic yield potential of maize (Selvaraj and Nagarajan, 2011). Plant height, days to tasseling, number of grains per row, number of grains per ear and grain weight recorded negative direct effect on grain yield even though genotypic correlation coefficients on grain yield were positive (Selvaraj and Nagarajan, 2011). Direct selection for ear length, ear height and number of rows per ear might be rewarding for yield improvement since they revealed true relationship with grain yield (Selvaraj and Nagarajan, 2011).

1.12 COMBINING ABILITY

General combining ability (GCA) is the average performance of a genotype in hybrid combination, while specific combining ability (SCA) is the condition in which certain combinations perform relatively better or worse than would be expected on the basis of average performance (Sprague and Tatum, 1942). The combining ability analysis is an important method to know gene actions and it is frequently used to select parents with a high GCA and hybrids with high SCA effects (Yingzhong, 1999). Information on combining ability is important for effective breeding strategies in a cross pollinated crops such as maize (Pavan et al., 2011).

Badu-Apraku and Oyekunle (2012) reported that GCA and SCA mean squares were significant for grain yield and most other traits under drought environments. Mean squares for GCA were larger than those of SCA in all environments, indicating that additive gene action was more important in the inheritance of traits (Badu-Apraku and Oyekunle, 2012). Badu-Apraku et al. (2011) reported that extra-early maize inbred lines are not only drought escaping but also possess genes for drought tolerance.

Lal et al. (2011) found that non-additive gene effects were important for silking date, anthesis date, plant height, ear height, ear length, grain yield per plant, number of rows per

ear, number of grains per row and grain weight. Premlatha and Kalamani (2009) also reported the predominant role of non-additive gene action for anthesis date, silking date, grain yield, ear length, number of rows per ear, number of grains per cob, number of grains per row, shelling percentage, harvest index, grain weight, cob girth and cob weight. Premlatha et al. (2011) also reported that combining ability analysis showed the predominant role of non-additive gene action for all the characters, and certain lines and testers were reported as good general combiners for a number of traits. Therefore, the parents may dominate each other in hybrid performance. Legesse et al. (2009) reported that GCA and SCA mean squares due to lines and testers were highly significant for grain yield and most of the studied traits. Reddy et al. (2011) reported significant mean squares due to GCA and SCA for grain yield and its components indicating that both additive and non-additive gene action, respectively, were important. However, the variances due to SCA were larger than GCA for all the characters indicating the predominance of non-additive gene action in the expression of various traits (Reddy et al., 2011).

Singh and Gupta (2008) used a line \times tester analysis in maize using twenty two lines and three testers under rainfed environment. The study compared nineteen characters including some morpho-physiological characters associated to drought tolerance. Among parents, five inbred lines were found to have negative GCA effects for anthesis date, silking date and days to maturity (Singh and Gupta, 2008). On the other hand five inbred lines revealed significant and positive GCA effects for grain yield and majority of the studied yield contributing traits (Singh and Gupta, 2008). Seven crosses for grain yield and some other traits revealed highly significant and positive SCA effects under water stress condition (Singh and Gupta, 2008). Non-additive gene effects were recorded for all the characters indicating that these characters can be exploited through hybrid breeding. In their study for combining ability over environments for twelve yield and yield related traits, Singh and Singh (2011) reported that the performance of lines, tester and crosses were significantly different in all the environments for all the traits except testers for number of leaves per plant. Inbred lines had good general combining ability for most of the traits (Singh and Singh, 2011).

1.13 LINE × TESTER ANALYSIS

Line x tester mating design provides a reliable information on the general and specific combining ability effects of parents and their hybrid combinations (Iqbal et al., 2007). Line x tester mating scheme is an efficient procedure because it allows for inclusion of a large number of lines and provides reliable estimates of genetic components, estimates combining ability and gene action governing quantitative traits (Sofi and Rather, 2006).

1.14 TESTERS

Li et al. (2007) reported that the choice of testers is important for evaluating combining ability, and defining heterotic groups and patterns of maize germplasm effectively and accurately. Guimaraes et al. (2012) reported that the choice of the most appropriate testers is important for a breeding program for ease of selection of the superior lines. This is supported by Russell (1961) who reported that an ideal tester should allow great expression of genetic variability in their progeny. Hence, the testers are important for determination of good lines. Hallauer and Carena (2009) also reported that the testers should be the best elite-lines of the breeding program, and new lines identified in superior crossings with the testers can be used in commercial hybrid development (Guimaraes et al., 2012).

1.15 CONCLUSIONS FROM THE LITERATURE

From the reviewed literature it is evident that stress is a major problem worldwide, especially in Africa, and it tends to depress maize production. Therefore, there lies a need for development of elite and affordable maize hybrids which are resistant to stress conditions especially drought stress tolerance without yield concession. There is a need to fully understand the nature of combining ability (CA) of parents (RILs and testers) involved in such hybrid development, and good CA for desirable traits between RILs and testers for desirable traits with emphasis on high grain yield is essential in a breeding programme. The literature showed that good CA has been reported for different secondary and adaptive traits with increased yield. Good genetic variation between lines and testers is essential for desirable hybrid development. For successful development of superior hybrids, associations among adaptive traits in hybrids involving maize RILs and testers need to be fully understood. The literature revealed that most desirable secondary and adaptive traits have

significant associations with grain yield, and this has implications for direct or indirect selection for grain yield and drought tolerance through such traits. The use of established and elite testers is essential for proper identification of superior lines which can be used for development of elite and stress tolerant hybrids.

1.16 REFERENCES

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ABSTRACT

The challenges posed by climate change and other environmental factors call for continuous development of new maize hybrids which are highly adaptable. Development of such hybrids starts with generation of good inbred lines. The objectives of the study were characterise recombinant inbred lines (RILs) using the augmented experimental designs. The 124 F₈ RILs were derived from an F₄ population following random mating to recombine genes at the F₂ and F₃ generations. The RILs were then characterised at the Ukulinga Research Farm during 2011/12. Standard cultural practices for maize were followed. The experiments were laid out as an augmented design. The data were analysed in GenStat 14th edition. Results indicated that the RILs were significantly ($p < 0.01$) different for all the traits suggesting that selection could be conducted to identify suitable parents for use in hybrids. A continuous distribution was observed for the following traits: ear position, anthesis date, ear height, and plant height and ear prolificacy percentage. However other traits such as ear prolificacy score, maize streak virus, grain texture and plant aspect scores did not show any continuous distribution, suggesting some involvement of a few genes or major quantitative trait loci (QTLs) for their determination. Cluster analysis based on nine phenotypic traits revealed that the parents are divided into two major groups, I and II. The cluster II comprises shortest inbred lines; while cluster I consists of tall, ear prolific, lodging resistant and good stand establishment parents. On the basis of the variation observed the RILs were used to make experimental hybrids. The concept of augmented experimental designs and their relative merits are reviewed.

Keywords: maize, recombinant inbred lines, augmented design, cluster analysis, skewness, continuous distribution, non-continuous distribution

2.1 INTRODUCTION

The development of inbred parents for hybrids is a very complex process which may take eight/nine seasons to achieve the desired level of homozygosity. The choice of parents is quite essential in the production of the maize hybrids (Zhang et al., 2002) because exploitation of heterosis in hybrids is highly reliant on the genetic background of the parents. In most cases new inbred lines are derived from bi-parental populations following segregation at the F_2 level. Thus the base population is created by crossing two complementary parents. New progeny lines which combine the desired traits from both parents are then targeted for selection in the F_2 generation and beyond. This process of selecting the desired lines requires an accurate field layout and proper experimental design to achieve a high level of precision to discriminate the lines according to their phenotypic traits in a classical plant breeding programme. The most common group of lines used in maize research are the recombinant inbred lines which are generated via classical approaches. The objectives of this chapter are to give an overview on the development of RILs, and to characterise the RILs, and to review the augmented designs. These RILs were used to generate hybrids which were evaluated using augmented designs in the subsequent research chapters.

2.2 RECOMBINANT INBRED LINES

The Recombinant Inbred Lines (RILs) are lines which are generated by inbreeding the F_2 progenies of a bi-parental crosses (Burr et al., 1988). Two parental inbred lines which are designated P_1 and P_2 , with alleles AA and BB for example, are crossed together to form a uniformly heterozygous F_1 generation which is advanced to F_2 , by self-pollination. F_2 progenies contain recombinant chromosomes due to crossovers between the two purely parental chromosomes present in each F_1 plant. Segregation of parental alleles occurs in the F_2 generation because it is a matter of chance just which of the three combinations of the alleles (A/A, A/B, or B/B) will occur in any of the F_2 progenies. Therefore the F_2 progenies are considered to be the founder parents of the RILs. The RILs are fixed through self-pollination following the single seed descent (SSD) method until F_8 . Each individual RIL will possess a different combination of recombinant and parental chromosomes, with an exclusive set of recombination breakpoint locations across the genome. A group of RILs form a segregant

QTL mapping population which can be maintained through SSD. The RILs can be used in genetic studies which can lead to development of new hybrids with better performance. This process is summarized in Figure 2-1. The RIL populations have been widely used in maize research. The Table 2-1 provides some examples of RIL populations that have been developed in maize. In the current study a modified approach was used to generate the RILs. This is described in the following sections.

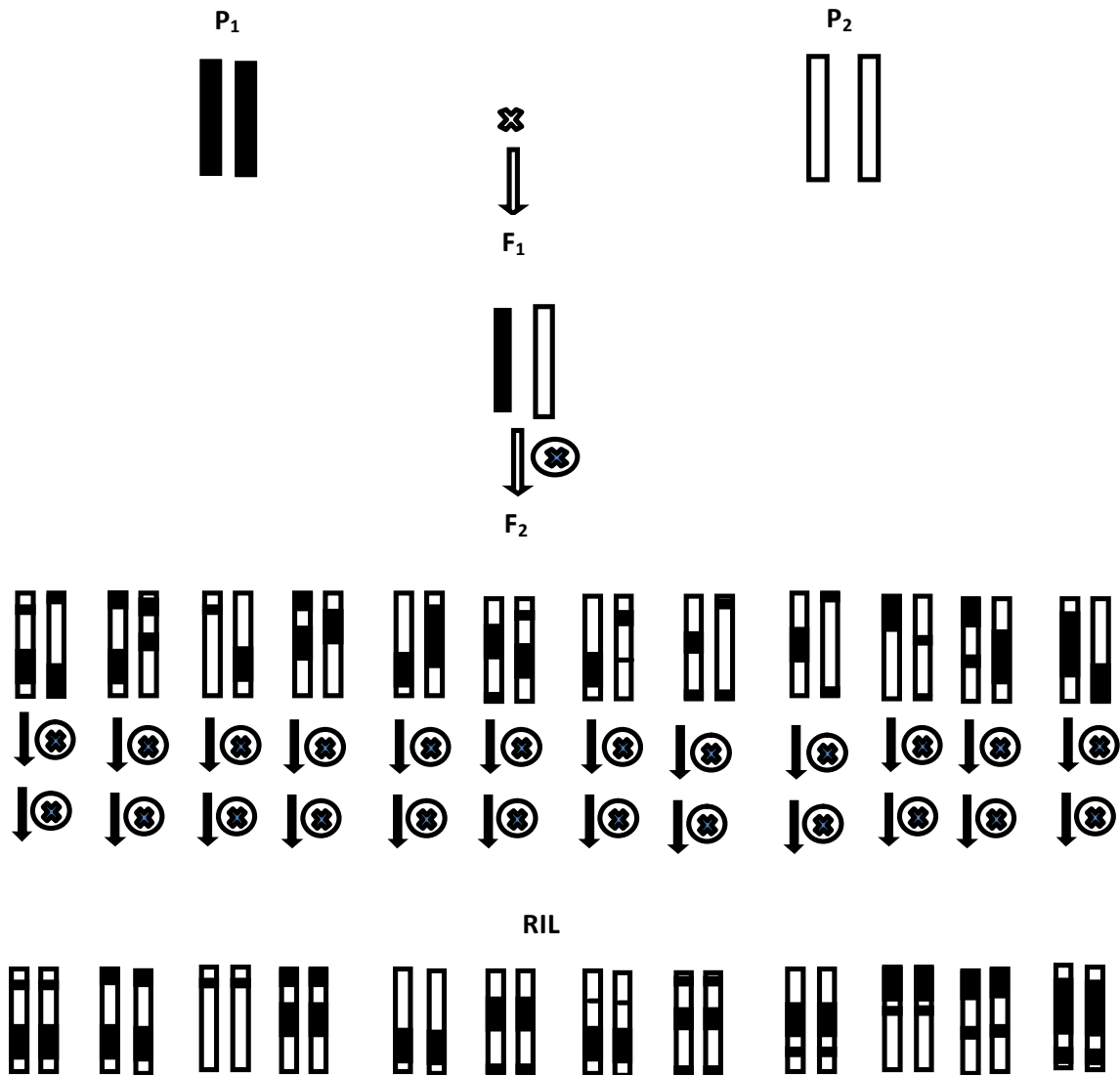


Figure 2-1: Outline for the production of RILs by successive self-pollination Source: (Xu, 2010).

Table 2-1: Some examples of RIL populations developed in maize (Burr et al., 1988)

Population	Population size
T232 × CM37	48
CO159 × Tx303	160
Mo17 × B73	44
PA326 × ND300	74
CK52 × A671	162
CG16 × A671	172
Ch593-9 × CH606-11	101
CO220 × N28	173

2.3 AUGMENTED DESIGNS

The statistical methods used for designing field trials need to be accurate and efficient (Federer and Crossa, 2012) which is reflected by the minimum error with implications for selection of the desired progeny lines. In plant breeding, there can be large number of new progenies to be evaluated and with few seeds each (Duarte and Vencovsky, 2005) resulting in complications for breeding. Consequently, Federer (1956) proposed the use of augmented experimental designs to deal with these challenges. According to Federer (1961) the augmented designs can be defined as any standard designs such as randomised complete block design and lattices which are augmented with additional treatments. The augmented designs contain a check or control variety which is replicated several times, and the experimental genotypes which are usually unreplicated in the experiment (Federer et al., 2001; Santos et al., 2002; Federer and Crossa, 2012). Therefore, augmented designs contain two kinds of treatments, standard check which is considered as fixed effects and augmented genotypes which are considered to be random effects (Federer et al., 2001). In this design, the checks are randomized according to a blocked design and blocks are filled up with experimental entries (Federer et al., 1975). The checks allow for an estimate of error and computation of adjusted means correcting for the incomplete block effects (Williams et al., 2011). Therefore, it becomes essential to use the checks which are adaptable to local

environments and which have known characteristics and performance. Importantly the checks have many seeds enabling replicated planting in all the blocks; whereas the experimental entries have very few seeds which are not adequate for replication.

The use of unreplicated experiments is not new in plant breeding because it is a cost effective operation which enables selection of the desired genotypes out of the bulk. Unreplicating experimental genotypes, and therefore, evaluating a larger number of genotypes, can bring better results to the plant breeding program (Peternelli et al., 2009). Federer and Crossa (2005) reported that augmented designs can be utilized to increase the efficiency of plant breeding programs. Augmented experimental designs can also be used in multilocation trials for hybrid or inbred line development where seed is not sufficient for planting more than one experimental unit at a single location (Federer and Raghavarao, 1975). These reports indicate that when a large number of genotypes is being evaluated it becomes unnecessary to replicate them, which thus emphasise the importance of augmented designs in such scenarios. In addition to seed, the land can also be limited when there are thousands of progenies to be evaluated in the breeding programme.

There are several classes of augmented designs which can be essential in controlling variability and assessing genotypes. According to (Federer and Crossa, 2012), these include the augmented block experimental designs, augmented complete block design, augmented row-column experimental designs, augmented incomplete-complete block design, augmented resolvable row-column, augmented split plot, and augmented split block experimental designs. Therefore maize breeders are presented with a wide option. The choice of each is highly dependent on the objectives and nature of the experiment. In the current study the augmented incomplete block design was used in the form of alpha lattice design. This is reported in the subsequent chapters (3, 4 and 5).

In choosing augmented designs breeders consider the advantages and disadvantages on the basis of operational and statistical reasons. An augmented design has many advantages such as : (i) more than one control can be used; (ii) standard errors of differences between experimental genotypes are available; (iii) standard errors of differences between experimental genotypes and checks are available; (iv) elite genotypes of a previous

screening season can be used as the checks for testing the new elite genotypes at the same time that a new set of genotypes are selected among them, (v) test entries need not be replicated which saves land space; and (vi) lesser cycles of selection are needed, hence cost and time are minimised (Federer et al., 2001; Kehel et al., 2010; Williams et al., 2011). They are also quite flexible experimental designs since the blocks can be unequal in size. There are also some disadvantages which may discourage the use of augmented designs, as follow: (i) there are few degrees of freedom for experimental error which thus negate the comparison among the treatments, but this can be alleviated by using many control varieties; (ii) unreplicated experiments are not fairly accurate, but they help breeders to narrow down on the entries showing promise which will be selected for replicated advanced trials; and (iii) considerable proportion of plots may need to be devoted to controls (Williams et al., 2011) which compromises the number of new genotypes to be tested. Given the foregoing, it is shown that augmented experimental designs are of utmost importance in maize breeding when seed and land are limited.

2.4 CHARACTERISATION OF RECOMBINANT MAIZE INBRED LINES

2.4.1 Germplasm development and study sites

A South African adapted single cross F_1 hybrid with 2 parents designated as P_1 and P_2 was developed. The F_1 was advanced to the F_2 by self-pollination and the seed was bulked at harvest. The F_2 seed was planted, self-pollinated and bulked to obtain F_3 . The process of advancing seed from F_1 to F_3 was done in Zimbabwe during 2004 to 2006. Individual plants from the F_3 were random mated using full-sib pollination to recombine genes at the Ukulinga Research Farm (Latitude = 29.66765 S; Longitude = 30.40602 E; and Altitude = 812 m.a.s.l) during the 2006/7 season. At harvest, the ears were shelled as individuals, and were planted ear to row at the Cedara Research Station (29.54192 S; 30.26494 E; and 1066 m.a.s.l) during the 2007/8. Then new lines were extracted from the F_4 families through self-pollination which was continued until the F_8 . This was achieved in a shuttle program between Makhathini Research Station (28.13663 S; 30.31514 E; and 1217 m.a.s.l) (winter season) and the Ukulinga Research Farm (summer). This represents a modification from the

traditional approach of developing RILs. Instead of using the single seed descent 15 seeds were selected from each ear and advanced to the next generation in each season.

2.4.2 Management of trials

The net plot length was 4 m, the distance between plants was 0.3 m, and the distance between the rows was 0.9 m. A total of 250 kg/ha NPK (56N: 83P: 111K) compound fertiliser was applied as basal dressing at planting. Six weeks after planting, 250 kg/ha of lime ammonium nitrate (LAN 28% N) was applied as a top dressing. The fields were kept clean of weeds using herbicides and hand weeding. The trials were rainfed. However, irrigation was applied after planting to establish the crop; afterwards the trials were rainfed until harvest.

2.4.3 Data collection

The following data were collected at Ukulinga and Makhathini Research Stations in accordance with protocols used by CIMMYT:

- Plant number: number of plants harvested per plot.
- Ear height (cm): measured as height between the base of a plant to the insertion of the uppermost ear of the same plant.
- Plant height (cm): measured as the distance between the base of a plant to the insertion point of the uppermost ear. It was measured when all the plants had flowered, since plants reach their maximum height at flowering.
- Number of ears: measured by counting the number of harvested ears per plot with one or more fully developed grain.
- Number of ears per plant: measured by counting the number of ears with at least one fully developed grain and divide by the number of harvested plants.
- Ear position (ratio): measured as the ratio of ear height to plant height. Small values less than 0.50 indicate a low ear position and large values (>0.50) indicate high ear position.
- Prolificacy percentage (%): measured by dividing number of prolific plants by the total number of plants harvested per plot and multiplied by 100.
- Maize streak virus (MSV): disease score on a 1-5 scale, where 1 is free from MSV disease and 5 is completely MSV infested.

- Turcicum leaf blight: disease score on a 1-5 scale, where 1 is free from turcicum disease and 5 is completely turcicum infested.
- Plant aspect: plant rating on a 1-5 scale, where 1 is excellently looking and 5 is very bad looking.
- Prolificacy score: ear prolificacy score on a 1-3 scale, where 1 means all the plants were prolific and 3 means all plants were not prolific.
- Grain texture: grain texture rating on a 1-5 scale, where 1 is flint and 5 is dent.
- Anthesis date (days): measured as the number of days after planting when 50% of the plants are shedding pollen.
- Root lodging (%): number of root lodged plants per plot at harvest divided by the total number of plants per plot and multiplied by 100.
- Stem lodging (%): number of stem lodged plants per plot at harvest divided by the total number of plants per plot and multiplied by 100.
- Total lodging (%): root lodged plus stem lodged plants per plot divided by the total number of plants per plot and multiplied by 100.

2.4.4 Data analysis

The data was analysed in GenStat 14th edition (Payne et al., 2011) to determine the means, phenotypic clusters and the frequency distribution of the parents based on phenotypic data. A dendrogram was used to determine the relatedness of the parents for different phenotypic traits for ease of selection of appropriate parents for the breeding program. Drinic' et al. (2012) reported that multivariate analyses such as cluster analysis are useful for measuring the degree of divergence among populations. Frequency histograms were plotted to determine the distribution of the parents for different traits. Pejic et al. (1998) assert that better understanding of genetic diversity assists breeders in planning crosses for hybrids and line development.

2.5 RESULTS

2.5.1 Agro-morphological variation among recombinant inbred lines

The means of all traits are presented in Table 2-2. The parents were significantly different ($p < 0.01$) for all the traits. The results for inbred line distribution for different traits are presented in Figure 2-2 and Figure 2-3. The results show that plant aspect, grain texture, turcicum, total lodging, maize streak virus and anthesis date were positively skewed. Whereas prolificacy percentage and number of plants per plot (stand establishment) were negatively skewed, while ear position, ear height, plant height and prolificacy score were normally distributed.

Table 2-2: Summary statistics for different traits of the recombinant inbred lines (n = 123) at Ukulinga Research Farm

Trait	Mean	± SE Mean	Min	Max	Variance	Probability
Ear height (cm)	58.0	± 1.27	21	98	198.2	<0.001
Plant height (cm)	154.4	± 1.900	92	220	443.9	<0.001
Ear prolificacy (%)	73.90	± 2.48	0.00	100	755.8	<0.001
Ear position (ratio)	0.37	± 0.01	0.19	0.51	0.004	<0.001
Number of plants	9.7	± 0.33	1.0	16.0	13.38	< 0.001
Number of ears per plot	16.7	± 0.58	1.0	28.0	41.9	< 0.001
Root lodging (%)	9.62	± 1.59	0.00	100	310.9	< 0.001
Stem lodging (%)	1.21	± 0.45	0.00	33.00	25.2	0.009
Total lodging (%)	10.82	± 1.61	0.00	100	320.5	< 0.001

SE Mean = standard error of mean; Min = Minimum; Max = Maximum

2.5.2 Clustering of recombinant inbred lines

The results are shown in Figure 2-4. The parents are grouped into two major clusters (I and II), cluster II is the largest. Cluster II is further subdivided into two clusters (A and B), where A is further divided into two clusters a_1 and a_2 .

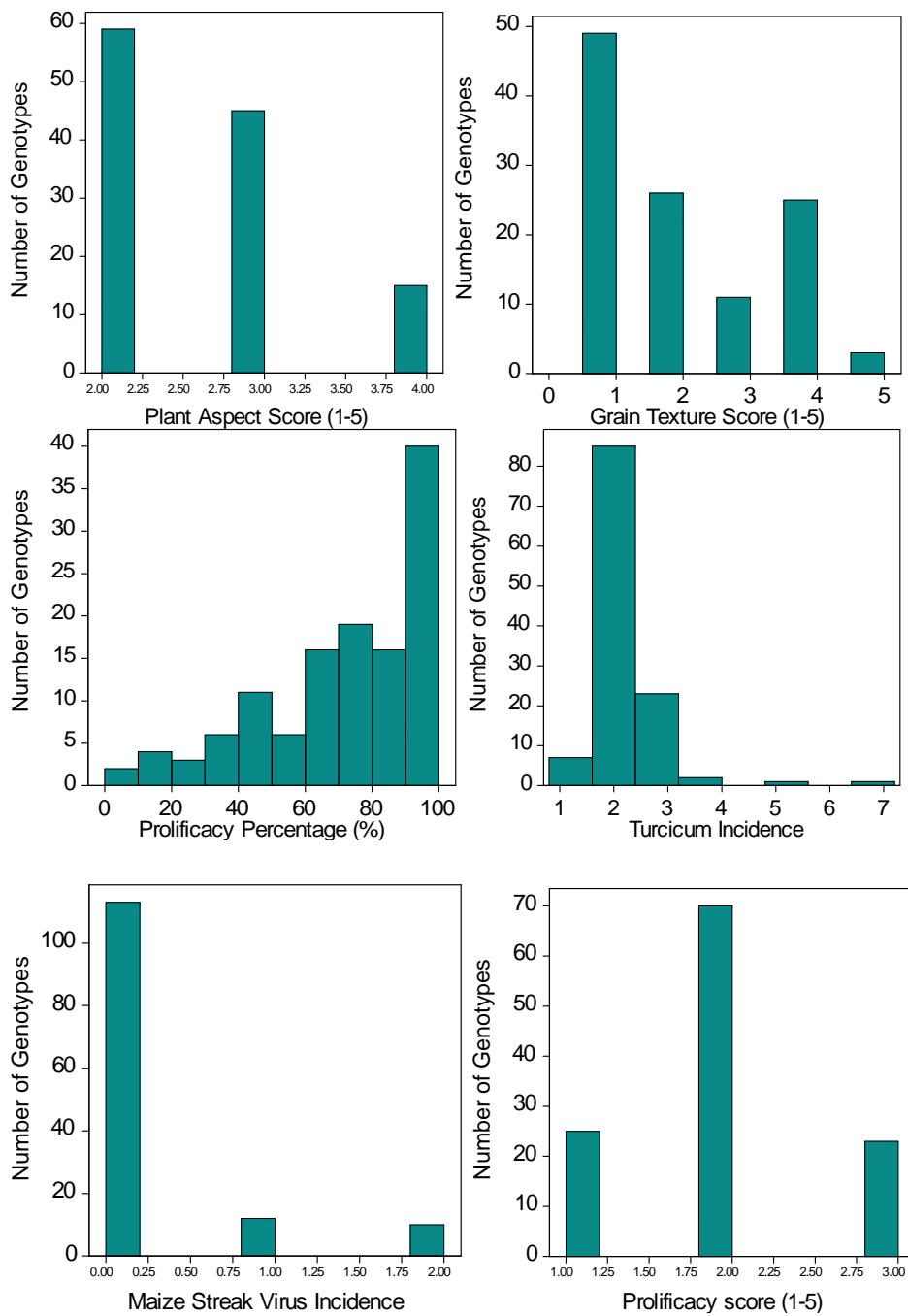


Figure 2-2: Histogram showing variation of the traits among maize parents

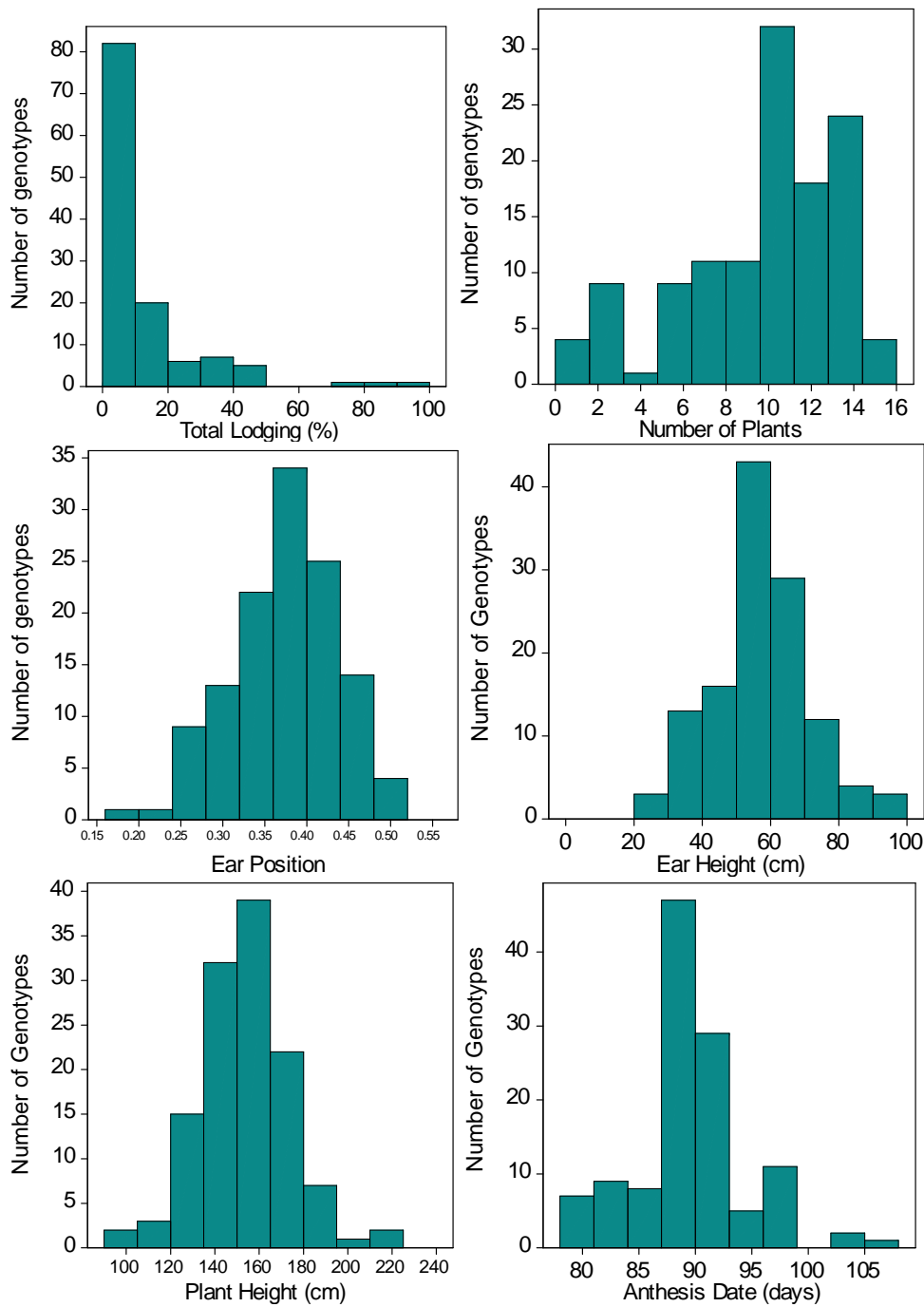


Figure 2-3: Histogram showing variation of the traits among maize parents

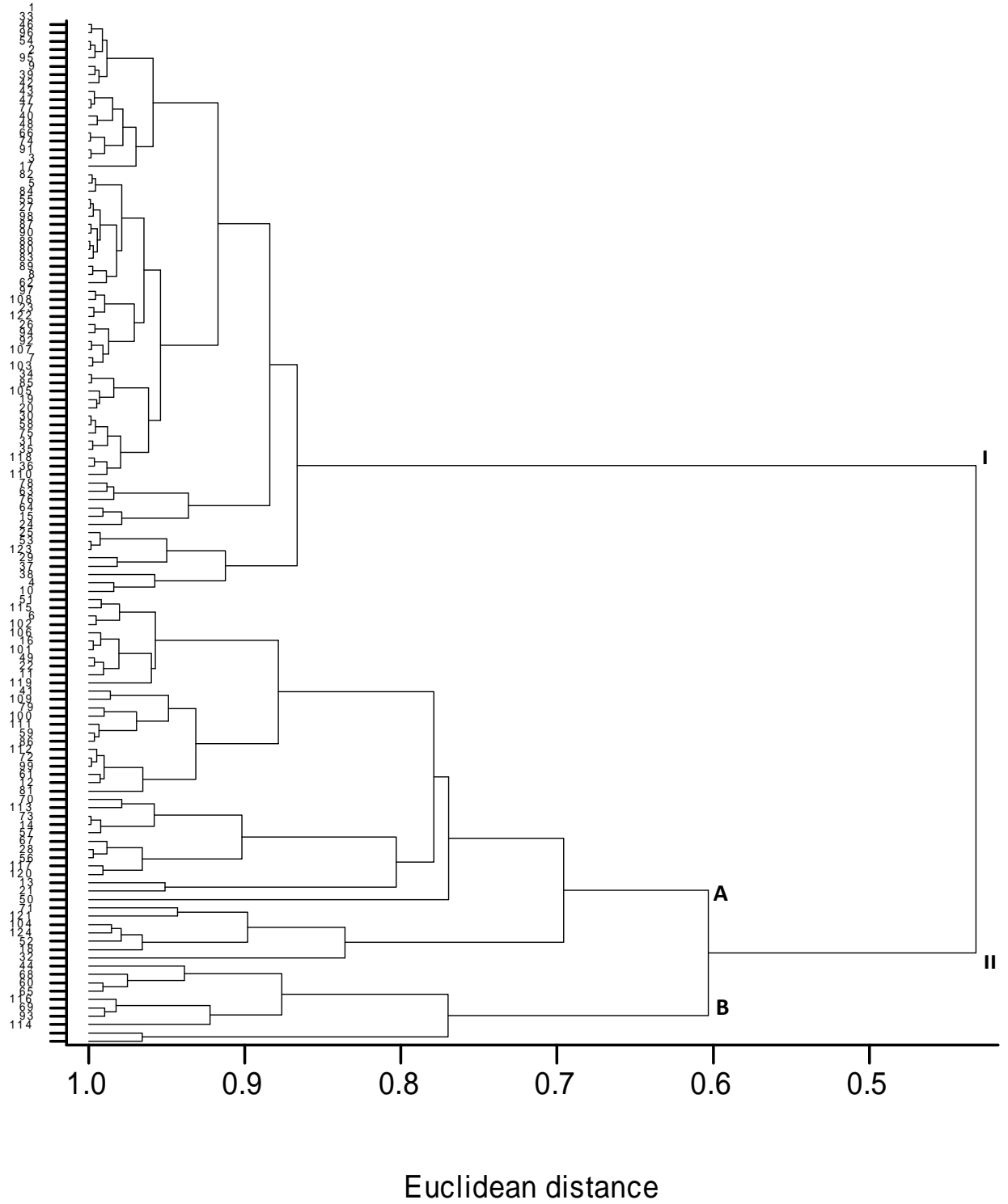


Figure 2-4: Dendrogram of 123 recombinant inbred lines for nine phenotypic traits based on Euclidean distance

2.6 DISCUSSION

2.6.1 Agro-morphological variation among recombinant inbred lines

There were highly significant differences among the RILs for all the traits. The results show that the parents performed differently from each other for all the traits, indicating that there was highly significant variation among the parents. Observation of this variation suggest that these RILs could be the ideal parents for the development of hybrids since they differed in phenotypic traits such as ear height, plant height, ear position, ear prolificacy, stand establishment and lodging. There are several authors who have reported on the related studies using different genotypes in different environmental conditions. The results from the current study are in accordance with Adeniyi (2011) who also reported significant results for plant height and related traits for different maize genotypes, where some genotypes were superior than others. Ahsan et al. (2011) also reported the significant differences among the maize inbred lines for various traits under stress conditions. These results indicate the variation in the performance of the genotypes which thus broaden the basis for selection of the desirable lines. Badu-Apraku and Akinwale (2011) also undertook a comparable study where they were identifying superior inbred lines for use as parents for hybrid development; indicating that it is important to evaluate the performance of the inbred lines before using them as parents in hybrid or line development.

In the current study, lower ear aspect score, turcicum and maize streak virus incidence, and ear prolificacy scores, and medium grain texture are desirable. Therefore RILs exhibiting these scores would be selected for use to make hybrids. Also higher ear prolificacy percentage is desired coupled with lower anthesis date and medium plant height, ear height and ear position, which are indicators of high yield, earliness, and resistance to lodging, respectively. The results show that plant aspect, grain texture, turcicum, maize streak virus, earliness and total lodging were positively skewed. These results show that the majority of the parents displayed the lower results for these traits, meaning that these parents could be relied upon for the selection of elite parents, since the majority of them displayed the lower results for these traits, which is desirable, except for grain texture where the medium score is desirable. Therefore, the majority of these parents still need some further improvement

for grain texture for ease of grain processing. The majority of the parents also displayed lower values for total lodging, suggesting that these results can also be used to select for lodging resistance since the majority of the parents were resistant to lodging. Ear prolificacy and stand establishment were negatively skewed, while ear position, ear height, plant height and prolificacy score were normally distributed. These results show that the majority of these parents were highly prolific and had high stand establishment, indicating that the majority parents could be used to breed for prolificacy and they also exhibited good stand establishment which is desired for higher grain yield. The results also show that the majority of the parents had the average ear position, ear height, plant height and prolificacy score; meaning that the parents exhibited the desirable plant stature and ear placement, and the majority of them can be used to breed for early maturity. The results further showed that the distribution was non-continuous for all the traits except ear position, ear height and plant height, suggesting that the majority of these traits are controlled by fewer genes or major QTLs implying the ease of selection and breeding for the majority of these traits. The results contrast Frova et al. (1999) who reported a normal distribution of all the genotypes for different traits. These results therefore contrast Holland (2007) who reported phenotypic traits to be controlled by fewer genes. An investigation of QTLs which control these traits in the RILs would be recommended. Overall significant variation among the RILs reflected the gains which were obtained by random mating the F_3 progenies before fixing of the lines through repeated self-pollination.

2.6.2 Clustering of recombinant inbred lines

When research resources are limiting it would be a good strategy to sample a few representative lines from the different clusters for developing hybrids; because lines in the same cluster are likely to share some alleles in common. The RILs were effectively divided into two major clusters: I and II, using phenotypic data. Cluster II comprise of short parents, whereas cluster I comprise of the tallest, prolific, lower lodging and good stand establishment parents. Therefore, the parents from cluster I can be used to breed for high ear prolificacy, lodging resistance, and hence higher grain yield. Cluster II is further subdivided into two clusters; A and B, where A is further divided into two clusters; a_1 and a_2 . Sub cluster B exhibited high ear prolificacy; however it was very susceptible to root lodging

whereas the opposite was witnessed for cluster A. Therefore, these parents still need further improvement for these traits, and this can be achieved by cross breeding them since they were highly variable from each other through these traits. Sub cluster a_1 had the shortest parents and they were least susceptible to lodging indicating superior standing ability; therefore this cluster can be used to select parents which could be used to incorporate lodging resistance in hybrids. The results from this study showed that the parents are highly variable from each other which provides ample opportunity to effect selection of RILs which are suitable for use in hybrid development. The results from the current study are in accordance with Ranatunga et al. (2009) who also reported that cluster analysis using 8 different qualitative traits across 43 maize genotypes resulted in grouping of genotypes into two major clusters. However, they are in contrast with Khodarahmpour (2012) who reported three major clusters for different maize genotypes under heat stress for grain yield and related traits. Generally, the results from different studies may not be comparable given that different sets of genotypes were tested under different environments using different set of traits. The observation from the current study also indicates that RILs in the same cluster can be random mated to enhance the concentration of the desired traits, in developing the second generation of superior lines from this population.

2.7 CONCLUSION

The objectives of the study were to give an overview on the development of parents of the hybrids which are used in the subsequent chapters. The study provides sufficient evidence that the RILs are sufficiently different and that there is adequate variation to justify selection of the best parents for hybrid development. The phenotypic performance results revealed that there are characteristic differences in the performance of parents which can thus be used to group these parents into different categories for ease of selection of desirable parents for hybrid development. Importantly, future breeding gains can be realised by random mating RILs in each of the two clusters to concentrate the desired alleles.

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ABSTRACT

Information on the nature of combining ability of parents, and their progeny performance in hybrid combination is crucial for maize breeding programmes. This study was aimed at selecting parental testers with best combining ability with recombinant inbred lines (RILs); and studying the relationship between grain yield and its components in hybrids. The RILs were crossed to 9 Zimbabwean tropical testers resulting in 1009 hybrids with sufficient seed for planting in trials. The hybrids were evaluated across four sites in South Africa during 2011/12 season in augmented alpha lattice design. A sample of 88 hybrids was selected for use in the study. The site main effects were highly significant ($p < 0.0001$) for all traits. The results indicated that the line GCA effects played a non-significant ($p > 0.05$) role in determining grain yield, grain moisture and anthesis date, while they were important for the other traits. The tester main effects were significant ($p < 0.05$) for all the traits except number of ears per plant and plant height. Line GCA effects were more important in conferring ear prolificacy and plant height in hybrids than the testers. On the basis of relative contribution the line main effects were predominant (30 to 54%) over the testers and SCA for all traits. The L1, 24 and 28 were the best general combiners for grain yield and prolificacy. Whereas tester T11 was the best general combiner for grain yield, prolificacy, and plant and ear height. There was the presence of both additive and non-additive gene actions in trait performance, where additive gene action had the most contribution to the traits. L114 x T12 had a significant and positive SCA effect for grain yield. This cross was identified for potential advancement in the next breeding programme. The correlation between yield and its traits was significant for prolificacy, grain moisture, ear height, plant height and anthesis date, indicating that indirect selection can be employed to enhance yield in South Africa by breeding for these particular adaptive traits. Plant height had the highest direct and indirect effect on grain yield. The study shows that productive hybrids can be developed using this set of RILs with tropical testers.

Keywords: additive gene action, correlations, general combining ability, maize, non-additive gene action, specific combining ability, Line x Tester analysis

3.1 INTRODUCTION

Maize yields in Africa are considerably lower than the world average because the cultivation of maize is often prone to drought and low soil fertility in addition to biotic stresses (FAO, 2011). Seed companies strive to produce newer hybrids and varieties with enhanced tolerance to stressful growing conditions (Bruce et al., 2002). To establish a sound basis for any breeding programme, aimed at achieving higher yield, breeders must have information on the nature of combining ability of parents, their behaviour and performance in hybrid combination (Chawla and Gupta, 1984, as cited by Bello and Olaoye, 2009; Pavan et al., 2011).

Therefore, the combining ability analysis is an important method to establish gene action governing traits such as high grain yield, secondary and adaptive traits. It is used for selection of the parents with a high GCA and hybrids with high SCA effects for desirable traits (Yingzhong, 1999). The study of the nature of combining ability is also useful to ascertain whether the traits are controlled by additive or non-additive gene action. Combining ability is often determined using line x tester mating design which provides a reliable information on the GCA and SCA effects of parents and their hybrid combinations, respectively (Iqbal et al., 2007). Mhike et al. (2011) reported that line x tester analysis is also useful for identification of the best testers, which is based on good GCA effects for major traits such as grain yield. In addition, the information on correlation studies among various plant traits is essential for establishment of the extent to which they are associated with yield, and they provide scope for indirect selection in a breeding programme (Yousuf and Saleem, 2001).

Therefore, the objectives of the study were to select testers and lines with best combining ability. It was hypothesised that the RILs are genetically divergent from the standard testers in the breeding program. The correlation between yield and its components also forms critical part of the study.

3.2 MATERIALS AND METHODS

3.2.1 Germplasm

The 118 RILs were developed as described in Chapter 2. The RILs were crossed to 9 Zimbabwean tropical testers resulting in 1009 hybrids with sufficient seed for planting in trials, and a sample of 88 hybrids with adequate seed for all the four sites were selected for the genetic analysis. Therefore 87 developmental hybrids and one commercial hybrid were used in the study. The commercial hybrid standard check (PAN6611) was obtained from PANNAR Seed Company in South Africa.

3.2.2 Experimental design and Management

The hybrids were evaluated across four sites in South Africa during 2011/12 season. The sites were Ukulinga Research Farm (Latitude = 29.66765 S; Longitude = 30.40602 E; and Altitude = 812 m.a.s.l), Cedara (29.54192 S; 30.26494 E; and 1066 m.a.s.l), Dundee (28.13663 S; 30.31514 E; and 1217 m.a.s.l) and Potchefstroom (26.73607 S; 27.07553 E; and 1349 m.a.s.l). The trials were planted on the 16th of November 2011 at Ukulinga, 11th of November 2011 at Dundee, 15th of December 2011 at Cedara, and 3rd of November 2011 at Potchefstroom. Experiments were laid out as augmented alpha lattice design. The test entries were not replicated but the control hybrid was replicated in each block. At the Ukulinga, Cedara and Dundee research stations the net plot length was 4 m, the distance between the plants was 0.3 m, and the distance between the rows was 0.9 m. At Potchefstroom research station, the net plot length was 6.8 m, the distance between the plants was 0.3 m, and the distance between the rows was 1.5 m. At all sites, 250 kg/ha NPK (56N: 83P: 111K) compound fertiliser was applied as basal dressing at planting. Six weeks after planting, 250 kg/ha of lime ammonium nitrate (LAN 28% N) was applied as a top dressing. The fields were kept clean of weeds using herbicides and hand weeding. The trials were rainfed at all sites. However, irrigation was applied after planting to establish the crop at Dundee and Potchefstroom; afterwards the trials were rainfed until harvest in June 2012.

3.2.3 Data Collection

The following data were collected at all the four sites in accordance with CIMMYT protocols:

- Grain yield ($t\ ha^{-1}$): measured by weighing the grain and ears and was adjusted to 12.5% grain moisture content.
- Number of ears per plant: measured by counting the number of ears with at least one fully developed grain and divided by the number of harvested plants
- Grain moisture content (%): measured as percentage water content of grain at harvest.
- Ear height (cm): measured as height between the base of a plant to the insertion of the uppermost ear of the same plant.
- Plant height (cm): measured as the distance between the base of a plant to the insertion point of the uppermost ear. It was measured when all the plants had flowered, since plants reach their maximum height at flowering.
- Ear position (ratio): measured as the ratio of ear height to plant height. Small values less than 0.50 indicate a low ear position and large values (>0.50) indicate high ear position.
- Plant number: number of plants harvested per plot.
- Anthesis date (days): measured as the number of days after planting when 50% of the plants are shedding pollen.

3.2.4 Data Analysis

The data was analysed using SAS version 9.2 (SAS Institute Inc, 2010) following a general linear model (GLM) procedure:

$$Y_{ijk} = \mu + S_i + G_j + \beta_k(S_i) + (G \times S)_{ji} + e_{ijk}$$

Where; Y_{ijk} = yield; μ = overall population mean; S_i = site; G_j = Hybrids (entries); $\beta_k(S_i)$ = blocks within sites; $(G \times S)_{ji}$ = Hybrid x Site Interaction; and e_{ijk} = Random experimental error.

The entries were fixed and blocks within sites, and sites x genotypes interaction were considered random. The genotype x environment interaction mean square was used as the error term to perform the F test for the hybrid effects.

The hybrid variation was partitioned into line and tester main effects giving two independent estimates of GCA effects, while the Line x Tester interaction effects estimate the specific combining ability (SCA). The model for Line x Tester analysis is as follows:

$$Y_{ijkl} = \mu + L_i + T_j + S_k + (L \times T)_{ij} + (L \times S)_{ik} + (T \times S)_{jk} + (L \times T \times S)_{ijk} + \beta_l(S_k) + e_{ijkl}$$

Where; Y_{ijkl} = observed genotype response; μ = overall mean; L_i = Line main effect; T_j = Tester main effect; S_k = Site main effect; $(L \times T)_{ij}$ = interaction between Line and Tester; $(L \times S)_{ik}$, $(T \times S)_{jk}$ and $(L \times T \times S)_{ijk}$ = interaction of Sites with Line, Testers and Line x Tester, respectively; $\beta_l(S_k)$ = Blocks within sites main effect; and e_{ijkl} = Random experimental error.

The GCA effects for parents were calculated according to Kearsey and Pooni (1996) as cited by Makanda (2009) and Makanda et al. (2009a) as follows:

$GCA_L = X_L - \mu$ and $GCA_T = X_T - \mu$, where: GCA_L and GCA_T = GCA of line and tester, respectively; X_L and X_T = mean of the lines and testers averaged over its crosses, respectively; μ = overall mean of all crosses.

The standard error (SE) for line and tester GCA effects were calculated according to Dabholkar (1999) separately because the numbers of males and females were not balanced as follows:

$SE_{Line} = \sqrt{MSE/S \cdot T}$, and $SE_{Tester} = \sqrt{MSE/S \cdot L}$, where: MSE = mean square error; S = number of sites; L and T = number of lines and testers, respectively.

The t-tests were calculated to determine the significance of lines, testers and line by tester interaction effects as follows:

$t_x = GCA_x / SE_x$, where: t_x = t-statistic of either line, tester or line x tester interaction analysis; GCA_x = general combining ability for either line or tester; and SE_x = standard error of either line or tester.

The SCA effects of the crosses were computed according to Kearsey and Pooni (1996) as cited by Makanda (2009) as follows:

$$SCA_x = X_x - E(X_x) = X_x - [GCA_L + GCA_T + \mu],$$

where: SCA_x = SCA effects of the two parents in the cross; X_x = observed mean value of the cross; $E(X_x)$ = expected value of the cross based on the GCA effects of the two parents; GCA_L and GCA_T = GCA of line and tester parents, respectively.

The standard error (SE) for the SCA effects were calculated according to Dabholkar (1999) as follows:

$$SE = \sqrt{MSE/S}, \text{ where: MSE = mean square error; and S = number of sites.}$$

The t-tests were calculated to determine the significance of lines, testers and line by tester interaction as follows:

$$t_x = SCA_x/SE_x; SCA_x = \text{specific combining ability for the cross.}$$

The Pearson's phenotypic correlation analysis was performed in SAS version 9.2 (SAS Institute Inc, 2010).

3.3 RESULTS

3.3.1 Variation among hybrids

The results are displayed in Tables 3-1 and 3-2. The site main effects were highly significant for all traits ($P < 0.0001$). The line main effects were significant ($P < 0.05$) for all the traits except grain yield, grain moisture and anthesis date. The testers' main effects were significant for all the traits except number of ears per plant and plant height. The interaction of line and tester effects was not significant for all the traits except plant height. The percentage sum of squares (% SS) of the Line and Tester GCA was greater than % SS SCA for all the studied traits; however, % SS Line GCA was greater than % SS Tester GCA for all the traits. Therefore, all the traits were mainly controlled by additive gene action where the lines had the most prominent contribution.

3.3.2 General combining ability of lines

The results for GCA effects of lines are presented in Table 3-3. In the current study, positive and significant GCA is desired for yield and prolificacy, while negative and significant GCA is desired for ear height, plant height, ear position, anthesis date and grain moisture content. Only those lines which displayed significant results for grain yield were explored for the other traits. There are seven lines (L1, 115, 104, 24, 28, 5 and 37) which displayed positive and significant GCA effects for grain yield. The L115 had the highest GCA effect, while 13 lines exhibited negative GCA effects for grain yield. Seven lines had significant and positive GCA effects for prolificacy. L24 had the highest GCA effect for prolificacy. Five lines had significant and negative GCA effects for grain moisture. Seven lines had significant and negative GCA effects for ear height. Eight lines had significant and negative GCA effects for plant height. The L10 had the lowest GCA effect for plant height. Four lines had significant

and negative GCA effects for ear position ratio. The L29 had the lowest GCA effects for ear position ratio. Eight lines exhibited significant and negative GCA effects for anthesis date.

3.3.3 General combining ability of testers

The results for GCA of testers are presented in Table 3-4. Two testers (T3 and T11) had significant ($p < 0.01$) and positive GCA effects for grain yield. T11 had the highest GCA effect for grain yield. Four testers had significant and negative GCAs for grain yield. Tester T12 had the lowest GCA effect for grain yield. Two testers had significant and positive GCA effects for number of ears per plant. Tester T12 had the highest GCA effect for number of ears per plant, followed by T11. Three testers had significant and negative GCA effects for grain moisture. Tester T12 had the lowest GCA effect for grain moisture. Four testers had significant and negative GCA effects for ear height. Tester T16 had the lowest GCA effect for ear height followed by tester T11. Three testers had significant and negative GCA effects for plant height. Tester T4 had the lowest GCA effect for plant height followed by T11. Two testers had significant and negative GCA effects for ear position ratio. Tester T16 had the lowest GCA effect for ear position ratio. Three testers had significant and negative GCA effects for anthesis date. Tester T12 had the lowest GCA effect for anthesis date.

Table 3-1: Primary traits mean squares and trial statistics for the maize crosses

Source	‡DF	Grain yield (t ha ⁻¹)	Ears per plant (no.)	Grain moisture (%)
Site	3	227.291 **	14.167 **	111.598 **
Cross	86	3.116 **	0.151 **	1.143 *
Line	41	2.498	0.155 **	0.918
Tester	8	10.016 **	0.159	3.898 **
Tester*Line	37	2.215	0.107	0.773
R ² (%)		75.0	78.6	99.6
CV (%)		26.1	20.0	6.1
Trial mean		5.51	1.45	14.25
Relative contribution				
% SS Line GCA		38.726	54.886	38.623
% SS Tester GCA		30.294	11.000	32.007
% SS SCA		30.980	34.114	29.370

*, ** Data significant at $P \leq 0.05$ and $P \leq 0.01$, respectively. ‡ = degrees of freedom were adjusted for the line x tester interaction.

Table 3-2: Secondary traits mean squares and trial statistics for the maize crosses across four sites

Source	†DF	Anthesis date (days)	Ear height (cm)	Plant height (cm)	Ear position (ratio)
Site	3	514.292 **	25624.881 **	76745.895 **	0.031 **
Cross	86	8.462 **	284.252 **	657.499 **	0.004 **
Line	41	5.168	268.197 **	649.480 **	0.003 *
Tester	8	20.145 **	494.293 **	630.071	0.006 **
Tester*Line	37	3.139	174.603	527.153 *	0.002
R ² (%)		82.2	84.7	85.3	55.3
CV (%)		2.5	9.9	8.0	8.8
Trial mean		75	115	236	0.487
Relative contribution					
% SS Line GCA		43.316	51.358	52.036	51.923
% SS Tester GCA		32.945	18.469	9.850	22.147
% SS SCA		23.740	30.173	38.114	25.930

*, ** Data significant at $P \leq 0.05$ and $P \leq 0.01$, respectively. † = degrees of freedom were adjusted for the line x tester interaction.

Table 3-3: GCA effects of selected maize inbred lines for grain yield and agronomic traits across four sites

Line	Grain yield t ha ⁻¹	Ears per plant no.	Grain moisture %	Ear height cm	Plant height cm	Ear position ratio	Anthesis date days
L1	1.05 **	0.15 **	1.20 **	12.44 **	6.53 *	0.04 **	3.67 **
L10	-0.90 **	-0.32 **	0.03	0.82	-19.47 **	0.05 **	2.00 **
L102	0.31	0.24 **	-0.16	-0.13	-5.47 *	0.01 *	0.87 *
L103	0.28	0.05	-0.31 *	-1.06	-7.72 *	0.01	-1.50 **
L104	0.72 **	0.22 **	0.06	-1.37	1.16	-0.01	0.50
L105	-0.43 *	-0.14 **	-0.07	-4.43 *	-7.72 *	0.00	-2.00 **
L106	-0.04	-0.08 *	0.27 *	4.47 *	7.13 *	0.00	1.34 **
L107	-0.39 *	-0.14 **	0.24	4.17 *	-7.07 *	0.03 **	0.60 *
L11	-0.94 **	-0.15 **	0.17	-1.68	-2.34	0.00	-0.66 *
L111	-0.17	0.02	-0.01	1.07	-9.80 **	0.03 **	0.45
L112	0.09	0.04	0.03	1.25	-0.28	0.01	-1.75 **
L113	-0.02	-0.16 **	-0.61 **	1.32	-12.97 **	0.03 **	0.67 *
L114	-0.15	0.19 **	-0.23	-1.18	3.16	-0.02 **	-0.50
L115	1.54 **	0.12 *	0.10	-0.93	-7.22 *	0.01 *	0.00
L117	-0.61 **	0.08 *	-0.77 **	-10.68 **	-17.13 **	-0.01	-0.55 *
L118	-0.08	-0.01	-0.37 *	4.57 *	7.48 *	0.00	0.40
L12	-0.50 *	-0.10 *	-0.10	0.32	1.03	0.00	-1.00 **
L13	-0.22	-0.04	0.50 **	-0.60	-4.05	0.00	1.56 **
L14	-0.85 **	-0.20 **	0.66 **	-21.43 **	-15.47 **	-0.06 **	-0.33
L15	-0.14	0.01	-0.08	-8.93 **	-5.22 *	-0.03 **	-0.33

Line	Grain yield t ha ⁻¹		Ears per plant no.		Grain moisture %		Ear height cm		Plant height cm		Ear position ratio		Anthesis date days	
L16	0.35		0.01		0.86	**	15.32	**	10.28	**	0.04	**	2.34	**
L17	0.09		0.05		0.43	**	-21.18	**	-1.22		-0.09	**	-2.00	**
L18	-0.78	**	-0.15	**	-0.21		-1.31		7.78	*	-0.02	**	-1.33	**
L19	0.28		-0.10	*	-0.04		1.65		13.53	**	-0.03	**	-1.33	**
L2	0.16		0.12	*	0.65	**	0.44		7.91	**	-0.01	*	1.50	**
L24	0.98	**	0.43	**	-0.67	**	13.82	**	26.28	**	0.01		2.67	**
L26	-0.01		-0.19	**	0.16		4.82	**	16.53	**	-0.01	*	0.00	
L28	1.09	**	0.18	**	0.00		8.32	**	13.78	**	0.01		3.34	**
L29	-0.54	*	0.32	**	0.13		-8.43	**	15.53	**	-0.07	**	4.00	**
L30	0.11		0.36	**	-0.53	**	6.82	**	29.53	**	-0.03	**	0.67	*
L37	0.52	*	-0.15	**	-1.07	**	6.57	**	11.53	**	0.00		-2.00	**
L4	-1.08	**	-0.38	**	0.40	*	-9.18	**	1.53		-0.04	**	-2.33	**
L44	0.35		-0.09	*	0.28	*	14.57	**	16.78	**	0.03	**	0.00	
L45	0.15		-0.07	*	0.03		-2.68		9.91	**	-0.04	**	-2.33	**
L46	-0.68	**	-0.16	**	-0.78	**	0.57		5.78	*	0.00		-0.33	
L47	-0.30		0.06		-1.19	**	-2.43		8.03	**	-0.03	**	0.00	
L48	-0.71	**	-0.08	*	0.85	**	0.32		-12.22	**	0.02	**	1.34	**
L49	-0.01		-0.16	**	-0.12		-3.68	*	-1.09		-0.02	*	-2.00	**
L5	0.42	*	0.03		0.35	*	-4.18	*	-1.47		-0.01		1.00	**
L50	-0.84	**	-0.17	**	-0.74	**	-10.68	**	-18.97	**	-0.01		-2.66	**
L51	-0.32		-0.25	**	0.56	**	3.82	*	-0.22		0.02	**	-2.00	**
L56	-0.12		0.04		-0.05		-9.93	**	-11.47	**	-0.02	**	0.67	*
SE	0.24		0.05		0.17		1.91		3.15		0.01		0.37	

*, ** Data significant at P ≤ 0.05 and P ≤ 0.01, respectively

Table 3-4: GCA effects of testers with their significance for grain yield and agronomic traits across four sites

Tester	Grain yield t ha ⁻¹		Ears per plant no.		Grain moisture %		Ear height cm		Plant height cm		Ear position ratio		Anthesis date days	
T12	-0.82	**	0.10	**	-0.49	**	-1.48	*	-3.89	**	0.00		-1.26	**
T4	-0.35	**	-0.02		-0.47	**	2.59	**	-9.49	**	0.03	**	-0.16	
T3	0.61	**	0.03		0.05		3.84	**	-2.09		0.02	**	1.34	**
T11	1.05	**	0.06	**	0.64	**	-3.52	**	-6.24	**	0.00		0.23	
T13	-0.33	**	-0.09	**	-0.17	*	-2.33	**	-1.82		-0.01	*	-0.46	**
T1	0.09		0.05	*	0.70	**	9.39	**	12.48	**	0.02	**	2.20	**
T14	0.06		-0.20	**	-0.12		-1.46	*	3.33	*	-0.01	**	-2.23	**
T15	-0.05		0.12	**	-0.16	*	0.89		8.38	**	-0.01	**	1.17	**
T16	-0.19	*	-0.05	*	0.10		-10.34	**	-1.62		-0.04	**	-1.00	**
SE	0.111		0.022		0.078		0.883		1.457		0.003		0.170	

*, ** Data significant at P ≤ 0.05 and P ≤ 0.01, respectively

3.3.4 Specific combining ability

The results for specific combining abilities are presented in Table 3-5. One cross (L114 x T12) had a significant and positive SCA effects for grain yield. Three crosses had significant and negative SCA effects for grain yield. Four crosses had significant and positive SCA effects for number of ears per plant. Cross L103 x T3 had the highest SCA effect for number of ears per plant. Two crosses had significant and negative SCA effects for grain moisture. Cross L112 x T3 had the lowest SCA effects for grain moisture. Six crosses had significant and negative SCA effects for ear height. Cross L107 x T3 had the lowest SCA effects for ear height. Four crosses had significant and negative SCA effects for plant height. Cross L104 x T14 had the lowest SCA effects for plant height. Six crosses had significant and negative SCA effects for ear position. Cross L104 x T12 had the lowest SCA effects for ear position. Nine crosses exhibited significant and negative SCA effects for anthesis date. Cross L1 x T1 had the lowest SCA effects for anthesis date.

3.3.5 Associations between traits

The correlation coefficients for grain yield data and secondary traits are presented in Table 3-6. Grain yield had highly significant ($p < 0.0001$) and positive correlations with all the traits except ear position and anthesis date. Number of ears per plant exhibited significant and positive correlations with ear height, plant height and ear position, but had significant and negative correlations with grain moisture and anthesis date. Grain moisture had a significant and positive correlation with plant height, but had significant and negative correlations with ear position. Ear height had significant and positive correlations with plant height and ear position, but exhibited a significant and negative correlation with anthesis date. Plant height displayed significant and positive correlation with ear position, whereas it exhibited a significant and negative correlation with anthesis date.

3.3.6 Path analysis

Path analysis results are presented in Table 3-7. Only the traits which displayed significant correlation with grain yield were used for path analysis. The results show that plant height, number of ears per plant and grain moisture had the highest direct and positive effects on grain yield, respectively. Whereas ear height and anthesis date had negative direct effects

on grain yield. The results also show that plant height had the highest indirect and positive effects on grain yield through ear height and number of ears per plant; whereas it had highly negative indirect effect on grain yield through anthesis date.

Table 3-5: SCA effects of crosses between lines and testers and their significance for different traits measured across four sites

Tester	Line	Grain yield t ha ⁻¹	Ears per plant no.	Grain moisture %	Ear height cm	Plant height cm	Ear position ratio	Anthesis date days
T3	L1	-0.61	-0.02	-0.03	-10.97 *	-7.66	-0.03	-1.00
T1	L1	-0.09	-0.05	-0.72	-2.27	-2.73	-0.01	-2.54 *
T12	L102	-0.69	-0.04	-0.09	-6.57	3.89	-0.04 *	0.06
T1	L102	-0.04	-0.37 **	0.14	0.81	9.77	-0.01	-1.74 *
T3	L103	0.57	0.38 **	-0.59	11.03 *	17.84 *	0.01	-0.84
T11	L103	-0.49	-0.14	0.56	-0.11	4.24	-0.01	2.94 **
T14	L103	0.08	-0.13	-0.21	-9.67 *	-11.83	-0.02	0.40
T12	L104	0.18	0.06	0.29	-1.83	4.52	-0.06 **	-0.24
T1	L104	0.51	0.23 *	-0.38	3.05	4.64	0.00	0.30
T14	L104	-0.91	-0.14	0.14	-9.85 *	-26.96 **	0.01	0.40
T12	L105	-0.12	-0.07	-0.03	3.48	-4.61	0.03	1.93 *
T14	L105	0.77	0.12	0.08	-3.79	10.67	-0.04 *	0.90
T4	L106	0.27	0.01	0.10	-0.99	15.89 *	-0.04 *	0.50
T3	L107	-0.31	-0.05	0.25	-12.69 *	-7.06	-0.04 *	1.40
T1	L11	0.30	0.02	0.04	-5.14	-0.61	-0.02	-1.87 *
T4	L111	-0.59	-0.29 *	0.80 *	-6.09	-2.93	-0.02	0.05
T12	L112	0.24	-0.09	0.94 *	-4.20	3.70	-0.03	2.35 *
T4	L112	-0.15	-0.12	0.83 *	2.22	-1.70	0.01	-1.42
T3	L112	-0.94	-0.01	-1.54 **	-0.53	12.15	-0.03	-0.92
T4	L113	0.35	0.02	0.47	-2.59	9.49	-0.03 *	0.16
T12	L114	1.52 *	-0.09	-0.52	5.73	7.27	0.01	-0.24
T11	L114	-1.76 *	-0.07	0.37	-0.73	2.87	-0.01	1.27
T3	L115	-1.30 *	-0.22	0.24	-9.59 *	-5.41	-0.03	-1.00
T11	L115	0.16	0.06	-0.88 *	3.02	12.24	-0.01	-0.89

Tester	Line	Grain yield t ha ⁻¹	Ears per plant no.	Grain moisture %	Ear height cm	Plant height cm	Ear position ratio	Anthesis date days
T4	L117	-0.07	0.12	-0.19	5.91	21.16 *	-0.02	-0.95
T16	L117	-0.01	-0.10	0.37	-5.66	-17.21 *	0.01	-0.11
T4	L118	0.58	0.12	-0.67	-7.59	-5.46	-0.03	-2.24 *
T3	L118	0.65	-0.25 *	0.58	-1.09	2.14	-0.01	0.26
T1	L118	-1.29 *	0.04	0.23	-7.14	-20.43 *	0.02	1.06
T15	L118	-0.48	-0.21	0.03	2.11	1.17	0.00	-1.90 *
T16	L118	0.45	0.16	-0.39	7.34	14.92 *	0.00	-0.74
T16	L12	0.19	0.05	-0.10	10.34 *	1.62	0.04 *	1.00
T1	L13	-0.02	-0.01	-0.05	1.53	-7.90	0.03	-2.09 *
T16	L14	0.19	0.05	-0.10	10.34 *	1.62	0.04 *	1.00
T16	L17	0.19	0.05	-0.10	10.34 *	1.62	0.04 *	1.00
T15	L18	0.01	-0.08	-0.15	-5.52	-16.38 *	0.01	-1.17
T16	L18	0.23	0.01	0.21	14.97 **	9.62	0.05 *	1.00
T14	L19	-0.55	0.03	-0.38	6.88	-5.83	0.05 *	0.23
T16	L19	1.02	0.22 *	0.10	-3.99	-5.13	-0.01	0.33
T1	L2	-0.64	-0.16	-0.50	-7.02	-13.11	0.00	-1.70 *
T15	L2	0.59	-0.01	-0.04	-3.27	-7.76	0.00	-1.67 *
T14	L26	-0.06	0.20	0.12	1.46	-3.33	0.01	2.23 *
T1	L28	-0.09	-0.05	-0.70	-9.39 *	-12.48	-0.02	-2.20 *
T14	L37	-0.06	0.20	0.12	1.46	-3.33	0.01	2.23 *
T14	L4	-0.06	0.20	0.12	1.46	-3.33	0.01	2.23 *
T14	L45	1.06	0.29 *	0.56	7.46	7.04	0.02	1.23
	SE	0.72	0.14	0.51	5.72	9.44	0.02	1.10

*, ** Data significant at $p \leq 0.05$ and $P \leq 0.01$, respectively

Table 3-6: Correlations between the maize traits in hybrids between RILs and testers over the four sites

Traits	Grain yield t ha ⁻¹	Ears per plant no.	Grain moisture %	Ear height cm	Plant height cm	Ear position ratio	Anthesis date days
Grain yield	1.000	0.514 **	0.352 **	0.607 **	0.711 **	0.060	-0.502 **
Ears per plant	0.514 **	1.000	-0.322 **	0.429 **	0.412 **	0.152 *	-0.434 **
Grain moisture	0.352 **	-0.322 **	1.000	0.019	0.270 **	-0.257 **	0.037
Ear height	0.607 **	0.429 **	0.019	1.000	0.871 **	0.588 **	-0.336 **
Plant height	0.711 **	0.412 **	0.270 **	0.871 **	1.000	0.125 *	-0.469 **
Ear position	0.060	0.152 *	-0.257 **	0.588 **	0.125 *	1.000	0.067
Anthesis date	-0.502 **	-0.434 **	0.037	-0.336 **	-0.469 **	0.067	1.000

*, ** Data significant at $P \leq 0.05$ and $P \leq 0.0001$, respectively

Table 3-7: Direct (underlined and bold) and indirect effects of grain yield components on grain yield across the four sites

Grain yield component	Ear height cm	Ears per Plant no.	Plant height cm	Grain moisture %	Anthesis date days	Total correlation to grain yield
Ear height	<u>-0.03</u>	0.17	0.42	0.00	0.04	0.60
Ears per plant	-0.02	<u>0.39</u>	0.20	-0.11	0.05	0.51
Plant height	-0.03	0.16	<u>0.48</u>	0.09	0.06	0.77
Grain moisture	0.00	-0.13	0.12	<u>0.35</u>	-0.01	0.34
Anthesis date	0.01	-0.16	-0.23	0.02	<u>-0.13</u>	-0.49

3.4 DISCUSSION

3.4.1 Variation among hybrids

The site main effects were highly significant for all traits; this shows the effect of environmental variation on plant performance. Each site has different environmental conditions which include latitude and altitude data which affect plant growth. Potchefstroom had the highest average rainfall, followed by Ukulinga, Cedara and Dundee; however, Dundee had the highest rainfall at flowering followed by Potchefstroom, Ukulinga

and Cedara. Potchefstroom had the highest average temperature, followed by Ukulinga, Dundee and Cedara; however, Ukulinga had the highest temperature at flowering, followed by Potchefstroom, Dundee and Cedara. These results show that different maize cultivars prefer different environmental conditions. These sites are suitable to determine the stability of maize genotypes across different environments in future studies. Mawere (2007) also reported significant differences for sites and entries in terms of grain yield in a different environment using different entries. In the future all the hybrids will be evaluated in many environments to identify the stable genotypes once the best set of hybrids have been isolated from the 1009 experimental hybrids that have been evaluated.

The line main effects were significant for all the traits except grain yield, grain moisture and anthesis date. The results indicate that the line GCA effects played a non-significant role in determining grain yield, grain moisture and anthesis date, while they were important for the other traits. The tester main effects were significant for all the traits except number of ears per plant and plant height. From the results it can be suggested that testers GCA effects had greater significant effect on grain yield, grain moisture and anthesis date than the line GCA effects. On the other hand the line GCA effects were more important in conferring prolificacy and plant height in hybrids than the testers. However, both line and tester GCA effects played a significant role in conferring ear height and ear position. The results contrast the results of Singh and Singh (2011) who reported non-significant differences in grain yield and related traits for different testers, however a different set of testers and lines was used in that study in a different environment. The interaction of line and tester effects was not significant for all the traits except plant height. The results show that SCA effects were not important for determining all the traits except plant height. Therefore, plant height was conditioned by genes with non-additive effects, and could be used to distinguish hybrids based on SCA data. The results are in contrast with Singh and Singh (2011) and Hussain et al. (2006) who reported significant SCA for grain yield and all related traits including plant height and suggested importance of non-additive gene action in conferring these quantitative traits in hybrids.

3.4.2 General combining ability of lines

The findings from the study underline existence of significant genetic variation among the maize RILs which can be exploited to develop new hybrids for possible deployment in South African maize belts. For this reason they will be recommended for use in designing hybrids which are suitable for the environments which are represented by the sites where the hybrids have been evaluated. There are seven lines which displayed positive and significant GCA effects for grain yield; this suggests that these lines produced above average grain yield when crossed with different testers across different environments. L115 had the highest GCA, which means that this line was best general combiner for grain yield. This line also exhibited significant and positive GCA effect for the number of ears per plant and significant and negative GCA for plant height. This behaviour is desirable because significant and positive GCA for number of ears per plant is a sign of prolificacy which is desirable for grain yield increment; whereas significant and negative GCA effect for plant height demonstrates that the line contributes additive genes for a short plant type which is desired. The line will be advanced in the programme where the main objectives are higher grain yield. There are also 13 lines which displayed significant and negative GCA effects for grain yield, and this is an indicator that these lines should be excluded from the breeding program where the main objective is grain yield; however, they can be evaluated for other agronomic traits. Alternatively, another set of testers should be identified for testing the potential of these lines.

The lines L1, 28 and 104 had significant and positive GCA effects for grain yield and prolificacy. These lines can be advanced in breeding program where the main objectives are higher grain yield and prolificacy; however, they still need to be improved for shorter plant height to reduce the risk for lodging. L24 had significant and positive GCA effects for grain yield and prolificacy, and it also displayed significant and negative GCA effect for grain moisture. It exhibited the highest GCA effect for prolificacy; therefore, this was the most prolific line. This line can be advanced in the breeding program where the main objective is higher grain yield coupled with prolificacy and lower grain moisture. Lower grain moisture is a sign of earliness and it also demonstrates that such plant can escape yield constraining stresses that occur at later stages during plant growth and it may not be prone to several

diseases which are usually favoured by increased moisture content in the kernels and the entire plant. L37 had the significant and lowest GCA effect for grain moisture, and it also displayed significant and positive GCA effect for grain yield and significant and negative GCA for anthesis date. The results show that this is the earliest line and it was also among the high yielders. Thus, this line has high utility and needs to be advanced in the breeding program where high grain yield and earliness are emphasised. L5 was also among the higher yielders and it also displayed lower ear height, since it exhibited significant and positive GCA effect for grain yield and significant and negative GCA effect for ear height. This line should also be among the lines which need to form part of the breeding program where higher grain yields and lower ear placement are major requirements. There are several lines which exhibited desirable combinations of traits; however, these lines did not display significant GCA effects for grain yield which thus suggest that these lines still require further improvements in their respective grain yield performance. L117 showed significant and positive GCA effects for prolificacy, and also displayed significant and negative GCA effects for grain moisture, ear height, plant height and anthesis date. This line has all the attributes of a desirable commercial hybrid (prolificacy, earliness, lower plant and ear height) except that it lacks significantly higher grain yield. This line needs to be improved for grain yield. It cannot be recommended for advancement in its current form.

The results from the current study are in accordance with previous findings by Mawere (2007), Bello and Olaoye (2009) and El-Badawy (2013) who reported significant GCA effects for grain yield and other parameters, in a different breeding programme using different entries and in different environments. Gebre (2005), Meseka et al. (2006) and Mhike et al. (2012) also reported significant differences for lines for grain. This significance of GCA effects indicate that additive effects contributed significantly to grain yield in hybrids, plant height, ear height and other traits under drought and non-drought environment.

3.4.3 General combining ability of testers

Results can reveal important information which can be used by the breeders in making effective use of this set of inbred testers from Zimbabwe, in developing new hybrids in combination with South African bred germplasm for possible deployment in Western and

Eastern South Africa. Therefore, the role and the behavior of each tester in hybrids are discussed. Two testers (T3 and T11) had significant and positive GCA effects for grain yield; these testers produced significantly higher yields when crossed with a number of lines. T11 had the highest GCA; this tester was the best combiner with different lines for increasing grain yield across four different sites. This tester also exhibited significant and positive GCA effects for other economic traits such as number of ears per plant and grain moisture content reflecting its utility for use in breeding for prolificacy and early maturity. This tester also exhibited significant and negative GCA effects for ear height and plant height, reflecting its superiority for use in breeding for good standing ability and plant stature. This is good because tall plants and high ear placements are not desirable traits for the environments in Western South Africa, since they increase the susceptibility of plants to lodging which can subsequently result into yield losses. This is the best tester as far as grain yield, prolificacy and short plant stature and ear placement are concerned, and it should form part of the base populations for the breeding programme where these traits are to be emphasised. However, it should not be considered in the breeding program where earliness is among the main objectives. For this reason the line should be improved for earliness through introgression of elite early lines from temperate environments.

Four testers (T12, T4, T13, and T16) had significant and negative GCA effects for grain yield; hence, these testers should be excluded from the breeding program where grain yield is the main objective. However, T12 can be used in the program where the main objective is earliness, since it displayed significant and negative GCA effects for grain moisture and anthesis date. This tester also displayed significant and positive GCA effects for prolificacy, significant and negative GCA effects for ear height and plant height. This tester had the desired attributes of the good tester except that it showed significant and negative GCA effect for grain yield under the environmental conditions in east and western South Africa. The tester T4 had significant and negative GCA effects for grain moisture and plant height signalling earliness and lower plant height, respectively. Even though the testers T12, T4, T13 and T16 had significant and negative GCA effects for grain yield they still possess the majority of desirable traits, therefore, they can be used in a breeding program where these traits are required and should be subjected to improvement for yield. Alternatively, these

lines can be recommended for use as donor lines for improving these traits in high yielding lines which are lacking in the traits which are contained by these testers. Importance of tester GCA effects for grain yield indicates that additive gene action is responsible for enhancing grain yield in their hybrids. These results are in accordance with Legesse et al. (2009), Kanagarasu et al. (2010) and Sadat et al. (2011) who also reported significant GCA effects of testers for grain yield and related traits. Worku et al. (2008) also reported significant GCA for number of ears per plant for testers.

3.4.4 Specific combining ability

Gene action results revealed that all the traits were controlled by both additive and non-additive genes, where additive gene action had the most contribution to the traits with implications for breeding new hybrids which are adapted in eastern and western maize belts in South Africa, and in similar environments elsewhere. The traits were controlled by additive gene action; because the percentage of the sum of squares for the Line GCA and Tester GCA were greater than those for the SCA for all traits. However, the % SS Line GCA was greater than % SS Tester GCA for all the traits meaning that in general the lines contributed more to the hybrids than the testers. Therefore, all the traits were mainly controlled by additive gene action where the lines had the most prominent contribution. The traits were also controlled by non-additive gene action because the crosses did not produce the expected results for all the traits based on the nature of GCA for the lines and testers. There was observation of a significant deviation from the expected.

One cross (L114 x T12) had a significant and positive SCA effect for grain yield, and it had the highest SCA effect for grain yield; this cross produced the highest grain yield whenever this line and tester were crossed together and grown across all four sites, and this was controlled by non-additive gene action, because both L114 and T12 had negative GCA effects but they had a positive SCA. This cross is going to be advanced in the breeding programme, and it needs to be further assessed for yield and yield parameters. Three crosses had significant and negative SCA effects for grain yield (L114 x T11, L115 x T3 and L118 x T1); these were the worst crosses as far as grain yield is concerned, whenever they were in combination grain yield would drastically decline regardless of the sites and their

environmental conditions. Cross L114 x T11 had the lowest SCA effect; this cross had the poorest specific combining ability of them all and it resulted into the lowest grain yield. This cross had parents which had differing combining abilities i.e. T11 which had highly significant and positive GCA effect and L114 which had non-significant and negative GCA; hence, these parents were not the good specific combiners for grain yield. Thus, this cross should be excluded in the breeding programme where grain yield is emphasized, but it can rather be assessed for other parameters. Even though the cross L115 x T3 displayed significant and negative SCA effect for grain yield, but it exhibited significant and negative SCA effect for ear height. Thus, this cross could be advanced in a breeding where the main objective is lower ear placement. L118 x T1 did not only show any significant and negative SCA effect for grain yield, but it also displayed a significant and negative SCA effect for plant height. Therefore, this cross can be advanced in a program where short plant height is desired.

Four crosses had significant and positive SCA effects for number of ears per plant; these crosses produced beyond average number of ears per plant across all four studied environments. Cross L103 x T3 had the highest SCA effect; this was the most prolific cross. These crosses should be evaluated in the program where the main objective is to improve prolificacy. Two crosses had significant and negative SCA effects for grain moisture; these crosses displayed very low grain moisture content. Cross L112 x T3 had the lowest SCA effect for grain moisture; this cross exhibited the lowest grain moisture content. These crosses should be used in the breeding program where low grain moisture content is the desired. Six crosses had significant and negative SCA effects for ear height. Cross L107 x T3 had the lowest SCA effect for ear height; this cross exhibited the lowest ear height across all the four sites. These crosses should be used in the program where lower ear height is the main objective. L107 x T3 also exhibited significant and negative SCA effect for ear position; hence, this cross could also be advanced in a breeding program where lower ear position is among the main objectives. L104 x T14 also displayed significant and negative SCA effect for plant height; therefore, this cross could be advanced in a breeding program where lower ear height and plant height are the main objectives. L28 x T1 also exhibited significant and negative SCA effect for anthesis date; thus, this cross can also be advanced in a program

where earliness is desired. Four crosses had significant and negative SCA effects for plant height. Cross L104 x T14 had the lowest SCA effect for plant height; this cross displayed the lowest plant height across all four environments. These crosses should be used in the breeding program where the main objective is lower plant height. Six crosses had significant and negative SCA effects for ear position. Cross L104 x T12 had the lowest SCA effect for ear position; this cross displayed the lowest ear position across all four environments. These crosses should be used in the breeding program where the main objective is lower ear position. Nine crosses exhibited significant and negative SCA effects for anthesis date. Cross L1 x T1 had the lowest SCA effect for anthesis date; this cross showed the lowest anthesis date across all four studied environments. These crosses should be used in the breeding program where earliness is the main objective.

The findings from the current study are consistent with previous reports that the SCA effects which indicate the role of non-additive gene effects are crucial in determining grain yield and its components. Alam et al. (2008) and Zivanovic et al. (2005) also reported significant SCA effects for grain yield. Zivanovic et al. (2005) reported that grain yield was more affected by non-additive genes, whereas Ojo et al. (2007) reported that additive gene action was more important than non-additive gene action for grain yield. Aguiar et al. (2003) reported that both additive and non-additive gene action were important for grain yield.

3.4.5 Associations between traits

Given the foregoing, it is prudent to discuss the relationships between grain yield and its component traits such as number of ears per plant, and also between the yield component traits among each other such as number of ears per plant, grain moisture, ear height, plant height, ear position and anthesis date. Grain yield had highly significant ($p < 0.0001$) and positive correlations with all the traits except ear position and anthesis date. These results show that grain yield increases with increase in all the studied traits except ear position and anthesis date. These traits should thus be further evaluated and re-assessed in the breeding programme comprising these hybrids. Anthesis date displayed a highly significant and negative correlation with grain yield. This behaviour shows that the earlier the anthesis dates the higher the grain yields. Number of ears per plant exhibited significant and positive

correlations with ear height, plant height and ear position like grain yield, but had significant and negative correlations with grain moisture and anthesis date. These results show that an increase in the number of ears per plant was coupled with increase in ear height, plant height and ear position, implying that selection for prolificacy was partly increasing plant height and ear placement. Perhaps additional internodes are required to produce additional ears, in the prolific genotypes. This behaviour of traits should be taken into consideration in the future breeding programme of these developmental hybrids because these traits viz., ear height, plant height and ear position have a certain limit (such as the ratio of 0.50 for ear position) that they should not exceed beyond which they can have detrimental effect on yield through lodging, unless if the stems are extremely callous. The results also show that increase in grain moisture and delayed anthesis date results in a decline in the number of ears produced by a plant which could negatively impact on yield. Therefore in designing new hybrids due care must be taken in defining the compromise for each trait.

Several previous studies have reported similar findings. For example, Guei and Wassom (1992) reported that number of ears per plant has a positive correlation with grain yield, indicating that yield can be enhanced through selection for prolificacy. Tan et al. (2006) and Umakanth and Khan (2001) reported that grain yield was significantly and positively correlated with plant height, meaning that in general yield was associated with tall plants with negative implication for breeding. Pradeep and Satyanarayana (2001) and El-Shouny et al. (2005) reported that grain yield was positively correlated with plant height and ear height. Harjinder et al. (2006) reported positive correlation between grain yield, plant height, ear height and number of ears per plant. On the one hand, Netaji et al. (2000) reported that grain yield was negatively correlated with anthesis date indicating that there could be challenges for improving grain yield in early maturing hybrids.

3.4.6 Path analysis

The results show that plant height had the highest direct effect on grain yield. Whereas ear height and anthesis date had negative direct effects on grain yield. The results also show that plant height had the highest indirect and positive effects on grain yield through ear height and number of ears per plant; whereas it had highly negative indirect effect on grain

yield through anthesis date. Therefore, plant height can be used to indirectly manipulate these traits in future breeding programmes. The results further show that even though anthesis date had an overall highly negative effect on grain yield, the majority of this effect was due to plant height, therefore plant height had a significant effect on the earliness of hybrids, indicating implications for indirect selection for these traits. These results contrast that of Abirami et al. (2007) and Gautam et al. (1999) who reported a directly low effect of plant height on grain yield. Mohammad et al. (2008) reported a highly positive direct effect of plant height on grain yield.

3.5 CONCLUSION

The objectives of the study were to select testers with best combining ability. The findings provide adequate evidence that the 9 testers from Zimbabwe are different as shown by their differences in combining ability for grain yield and its components in the South African environments. The best tester identified is T11 which showed outstanding combining ability for grain yield with the RILs in addition to other economic traits.

It was confirmed that the RILs are genetically divergent from the standard testers in the breeding program, and are complementary in forming hybrids, because the lines contributed significantly to prolificacy while the testers were significant for grain yield enhancement and earliness. The study also confirmed that there is adequate genetic variation among the lines which provides the opportunity for selection of the most appropriate RILs to make new hybrids.

The correlation between yield and its components was significant for the prolificacy, grain moisture, ear height, plant height and anthesis date, indicating that indirect selection can be employed to enhance yield in South Africa by breeding for these particular adaptive traits. Therefore it can be concluded that there is a significant correlation between adaptive traits and productivity in the recombinant inbred lines (RILs) with implications for devising the most appropriate breeding strategy. Importantly, results also reveal some weaknesses of both the RILs and testers which provide the opportunity for further breeding.

Path analysis revealed that plant height was the most important trait both directly and indirectly for grain yield increment. Therefore, this trait can be used in future breeding programmes for grain yield enhancement.

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Chapter 4: Genetic variation and associations among adaptive traits in hybrids involving recombinant maize inbred lines

ABSTRACT

Maize breeding begins by genetic variability determination of the base population, and studying associations between grain yield and its adaptive traits. Therefore, the objectives of this study were to determine genetic variation and associations among adaptive traits in hybrids involving recombinant inbred lines (RILs). The 118 RILs were derived from a South African F_3 bi-parental population using classical pedigree breeding method, and self-pollination to advance the generations to the F_8 . The RILs were crossed to 9 Zimbabwean tropical testers resulting in 1009 hybrids. The hybrids were evaluated across four sites in South Africa during 2011/2012 season where the experiments were laid out as augmented alpha lattice design. All quantitative data was subjected to GenStat and SAS statistical softwares. Phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the traits. All the traits displayed high heritability at Potchefstroom except anthesis which was highly heritable at Ukulinga. Cedara was the second best site for heritability of all the traits except for the number of ears per plant. The genetic advance for grain yield was the highest at Cedara followed by Potchefstroom, Dundee and Ukulinga. The hybrids exhibited different patterns of variations for all the traits. The distribution was continuous for all the traits in all the sites except anthesis date and plant height at Potchefstroom. There were significant phenotypic correlations between grain yield and its adaptive traits, and among adaptive traits. Ear length had the highest direct effect on grain yield at Ukulinga; number of ears per plant had the highest direct effect on grain yield at Cedara and Potchefstroom; whereas plant height had the highest direct effect on grain yield at Dundee. Grain yield was least affected by indirect factors in all the sites except Ukulinga, where anthesis date had the highest indirect effect on grain yield through silking date followed by plant height through leaf area. Therefore, indirect selection for anthesis date and plant height at Ukulinga and similar environments can be employed.

Keywords: Genotypic coefficient of variation, maize, path coefficient analysis, phenotypic correlation, phenotypic coefficient of variation, recombinant inbred lines

4.1 INTRODUCTION

Maize breeding begins by the genetic variability determination of starting breeding material (Babic' et al., 2011). Babic' et al. (2011) also reported that genetic divergence of parental inbred lines is the main step to get high heterotic effect in yield after crossing. While Govindaraj et al. (2010) also emphasized that genetic variability for agronomic traits is the key component of breeding programmes.

Therefore, knowledge of genetic variation is fundamental in breeding programmes, because the plant populations and varieties vary at the genetic level and as a result they have differing phenotypic performance. Thus it is essential to know how variable the populations of interest are so that it can be easy to construct and plan an ideal genotype. During the evaluation of breeding material genetic advance is usually determined. Genetic advance shows the degree of gain obtained in a character under a particular selection pressure (Bello et al., 2012). Before introgression of genes coding for a trait of interest, it is essential to determine its heritability. Therefore, it is necessary to partition the observed variability into its heritable and non-heritable components and to have an understanding of parameters such as genetic coefficient of variation, heritability and genetic advancement (Govindaraj et al., 2010). Heritability is a quantitative measure which provides information about the proportion of genotypic variance out of the total phenotypic variance (Dabholkar, 1999). Heritability can be classified into broad and narrow sense (Gebre, 2005). In most instances a large percentage for a character is regarded as highly heritable (Dabholkar, 1999). The knowledge of heritability can also be useful in identifying how much of an adaptive trait has been transferred into the successive generation.

Yousuf and Saleem (2001) reported that information on correlation among plant traits is important for determination of the degree to which they are associated with yield. The association studies are also essential since they have implications on indirect selection for grain yield, hence it is essential to determine the nature of impact that a particular trait has on yield. Sometimes these traits can affect grain yield through other traits. Therefore there is a need to study path coefficient analysis. Path coefficient analysis provides an effective means of partitioning correlation coefficients into unidirectional pathways and alternate

pathways thus permitting a critical examination of specific factors that produce a critical correlation which can be successfully employed in formulating an effective selection programme in breeding (Salahuddin et al., 2010). According to Makanda et al. (2009b) path coefficients give the relative contribution of secondary traits, enabling breeders to decide between direct and indirect selection. It is essential to know whether the trait you are working on has a direct or indirect positive or negative effect on overall plant yield.

The objectives of this chapter were to determine genetic variation and associations among adaptive traits in maize hybrids involving recombinant inbred lines in each location.

4.2 MATERIALS AND METHODS

4.2.1 Germplasm

The development of germplasm under study is described in chapters 2 and 3.

4.2.2 Experimental design and Management

The trials were designed and managed as reported in chapter 3. The rainfall data from November 2011 to April 2012 for all the sites is displayed in Figure 4-1. On average Potchefstroom had the highest rainfall, followed by Ukulinga, Cedara and Dundee, respectively. The temperature data from November 2011 to April 2012 is presented in Figure 4-2. On average Potchefstroom had the highest temperature, followed by Ukulinga, and Cedara, respectively.

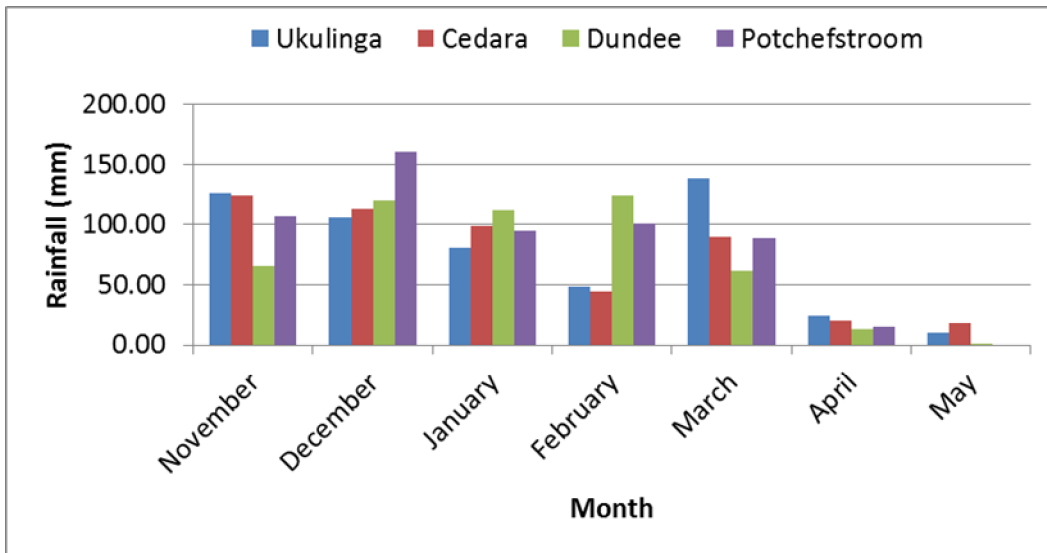


Figure 4-1: Rainfall amount of the four sites from November 2011 to May 2012

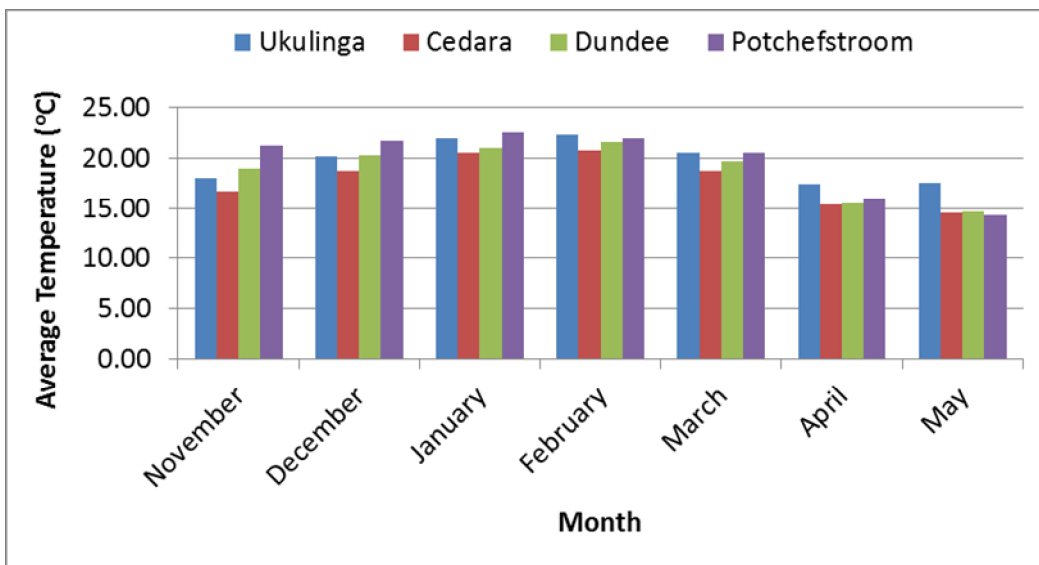


Figure 4-2: Average temperature of the four sites from November 2011 to May 2012

4.2.3 Data Collection

The following data were collected at all the four sites in accordance with protocols used by CIMMYT:

- Grain yield (t ha^{-1}): measured by weighing the grain and ears and was adjusted to 12.5% grain moisture content.
- Number of ears per plant: measured by counting the number of ears with at least one fully developed grain and divide by the number of harvested plants
- Grain moisture content (%): measured as percentage water content of grain at harvest.
- Ear length (cm): measured as the length of the bigger and longer ear (potential ear length).
- Ear height (cm): measured as height between the base of a plant to the insertion of the uppermost ear of the same plant.
- Plant height (cm): measured as the distance between the base of a plant to the insertion point of the uppermost ear. It was measured when all the plants had flowered, since plants reach their maximum height at flowering.
- Ear position (ratio): measured as the ratio of ear height to plant height. Small values less than 0.50 indicate a low ear position and large values (>0.50) indicate high ear position.
- Plant number: number of plants harvested per plot.
- Anthesis date (days): measured as the number of days after planting when 50% of the plants are shedding pollen.
- Silking date (days): measured as the number of days after planting when 50% of the plants produced silks.
- Anthesis-silking intervals (ASI) (days): $\text{ASI} = \text{SD} - \text{AD}$.
- Number of tassel branches: measured after pollination as the number of tassel branches excluding the primary tassel.
- Kernel rows per ear: counted as number of kernel rows in the central part of the uppermost ear.
- Number of kernels per row: counted as the number of kernels in the longest row (potential).
- Number of kernels per ear: measured by multiplying number of rows per ear by number of kernels per row.
- Number of leaves: measured by counting all leaves visible on a plant, whether rounded or pointed and whether collared or not.

- Chlorophyll content (CCI): measured at a week interval post-silking for two growth stages (weeks) on the uppermost ear leaf using the chlorophyll meter.
- Ear leaf area (cm²): measured as the area of the ear leaf of the uppermost cob.
- GLS and PLS (score): disease score on a 1-5 scale, where 1 is free from disease and 5 is completely disease infested.
- Ear aspect (score): cob rating on a 1-5 scale, where 1 has desirable ear aspect and 5 has poorest ear aspect.

4.2.4 Data Analysis

The data was analysed using SAS version 9.2 (SAS Institute Inc, 2010) following a general linear model (GLM) procedure:

$$Y_{ijk} = \mu + S_i + G_j + \beta_k(S_i) + (G \times S)_{ji} + e_{ijk}$$

Where; Y_{ijk} = yield; μ = overall population mean; S_i = site; G_j = Hybrids (entries); $\beta_k(S_i)$ = blocks within sites; $(G \times S)_{ji}$ = genotype x site interaction, which was considered as random; and e_{ijk} = Random experimental error. The GxS interaction mean square was used as the error term to the F-test for the across site analysis.

The histograms displaying the distribution of hybrids for each trait were generated using GenStat 14th Edition (Payne et al., 2011). All quantitative data was subjected to analysis of variance, using GenStat and SAS statistical softwares. The genotypic (δ^2_g), phenotypic (δ^2_p) and error (δ^2_e) variances were computed, using the REML tool in GenStat. The genotypic (δ^2_g), phenotypic (δ^2_p) and error (δ^2_e) variances were estimated using the formulae of Burton and De Vane (1953; as cited by Bezawelelaw et al. (2006)) as $\delta^2_g = (MSg - MSe)/r$; $\delta^2_p = \delta^2_g + \delta^2_e$ and $\delta^2_e = MSe$, where MSg = genotypic mean square, MSe = environmental variance (error mean square) and r = the number of replications. The phenotypic coefficient of variance (PCV), genotypic coefficient of variance (GCV) and error coefficient of variance (ECV) were estimated following the procedure of Kumar et al. (1985; as cited by Bezawelelaw et al. (2006)): $PCV = 100(\delta_p)/\bar{X}$; $GCV = 100(\delta_g)/\bar{X}$ and $ECV = 100(\delta_e)/\bar{X}$, where δ_p = phenotypic standard deviation, δ_g = genotypic standard deviation, δ_e = environmental standard deviation and \bar{X} = character mean. Heritability (h^2) in a broad sense was estimated by the formulae of Allard (1960; as cited by Bezawelelaw et al. (2006)): $h^2 = \delta^2_g/\delta^2_p$. Genetic advance (GA) values were determined as described by Burton (1952). $GA = (K)(\delta)(H^2)$;

where, $K = 2.063$ (selection differential at 5%), δ = phenotypic standard deviation of the mean yield of n original lines and H^2 = broad sense heritability. The Pearson's phenotypic and genetic correlation analysis was performed in GenStat 14th Edition (Payne et al., 2011) and SAS version 9.2 (SAS Institute Inc, 2010). Path analysis was performed in SAS version 9.2 (SAS Institute Inc, 2010), following the Cramer and Wehner (2000) procedure. Path-coefficient analysis was conducted to estimate the relative contribution of various yield-determining traits, enabling breeders to decide between direct and indirect selection.

4.3 RESULTS

4.3.1 Genetic variation, heritability and genetic advance of selected traits across four different environments

Results indicated significant differences between genotypes at all the sites. The genetic parameters are presented in Table 4-1. Grain yield heritability ranged between 27 and 70% across all the sites, whereas its genetic advance ranged between 21 and 29%. Number of ears per plant heritability ranged from 30 to 53%, while its genetic advance ranged between 15 and 24%. Anthesis date heritability ranged between 53 and 65%, but its genetic advance ranged between 2 and 6%. Ear height heritability ranged between 18 and 87%, although its genetic advance ranged between 7 and 14%. Plant height heritability ranged between 29 and 81%, where its genetic advance ranged between 6 and 11%. Ear position heritability ranged between 24 and 76%, though its genetic advance ranged between 7 and 18%.

Table 4-1: Genetic parameters of selected traits of 1009 hybrids across four different sites

Genetic Parameter	Ukulinga						Cedara					
	Yield	EPP	AD	EH	PH	EPO	Yield	EPP	AD	EH	PH	EPO
Mean	4.02	1.0	78	114	230	0.50	8.12	1.4	73	130	272	0.48
VG	0.491	0.014	5.127	101.900	139.600	0.002	1.541	0.040	4.603	132.300	210.280	0.001
GCV (%)	17.43	11.48	2.92	8.85	5.15	10.02	15.30	2.94	14.72	8.83	5.34	6.57
PCV (%)	30.82	16.56	3.64	13.24	9.51	11.61	23.56	3.81	26.64	12.94	6.25	11.21
H² (%)	31.99	48.02	64.34	44.63	29.26	74.49	42.15	30.53	59.68	46.54	72.89	34.35
GA	0.889	0.173	4.228	15.677	14.731	0.090	1.714	0.234	3.502	16.268	26.908	0.038
GAM (%)	22.11	17.30	5.42	13.75	6.40	18.00	21.10	16.71	4.79	12.51	9.89	7.91
	†Potchefstroom						Dundee					
Mean	6.86	2.1	73	135	259	0.52	4.06	1.3	-	83	188	0.44
VG	0.663	0.041	1.087	107.680	154.960	0.001	0.834	0.054	-	40.200	191.300	0.001
GCV (%)	11.87	9.61	1.42	7.71	4.80	6.03	22.48	17.23	-	7.64	7.34	6.88
PCV (%)	14.28	13.23	1.93	8.29	5.33	6.95	42.77	30.88	-	17.64	12.44	13.88
H² (%)	69.13	52.70	53.98	86.53	81.03	75.27	27.63	31.13	-	18.76	34.85	24.59
GA	1.649	0.330	1.582	18.984	22.252	0.054	1.161	0.312	-	6.179	20.163	0.031
GAM (%)	24.03	15.71	2.16	14.06	8.59	10.38	28.59	24.00	-	7.44	10.72	7.04

† Only 132 hybrids were evaluated at Potchefstroom. EPP = number of ears per plant. AD = anthesis date. EH = ear height. PH = plant height. EPO = ear position. VG = genetic variation. GCV = genotypic coefficient of variation. PCV = phenotypic coefficient of variation. H² = heritability. GA = genetic advance. GAM = genetic advance as a mean percentage.

4.3.2 Variation of hybrids for grain yield and associated traits

The distribution of hybrids at Ukulinga is presented in Figure 4-3. The data exhibited some positive skewness of hybrids for grain yield, number of ears per plant, grain moisture, anthesis date and ear position. Ear height showed normal distribution; whereas plant height exhibited negative skewness. The distribution was continuous for all the traits.

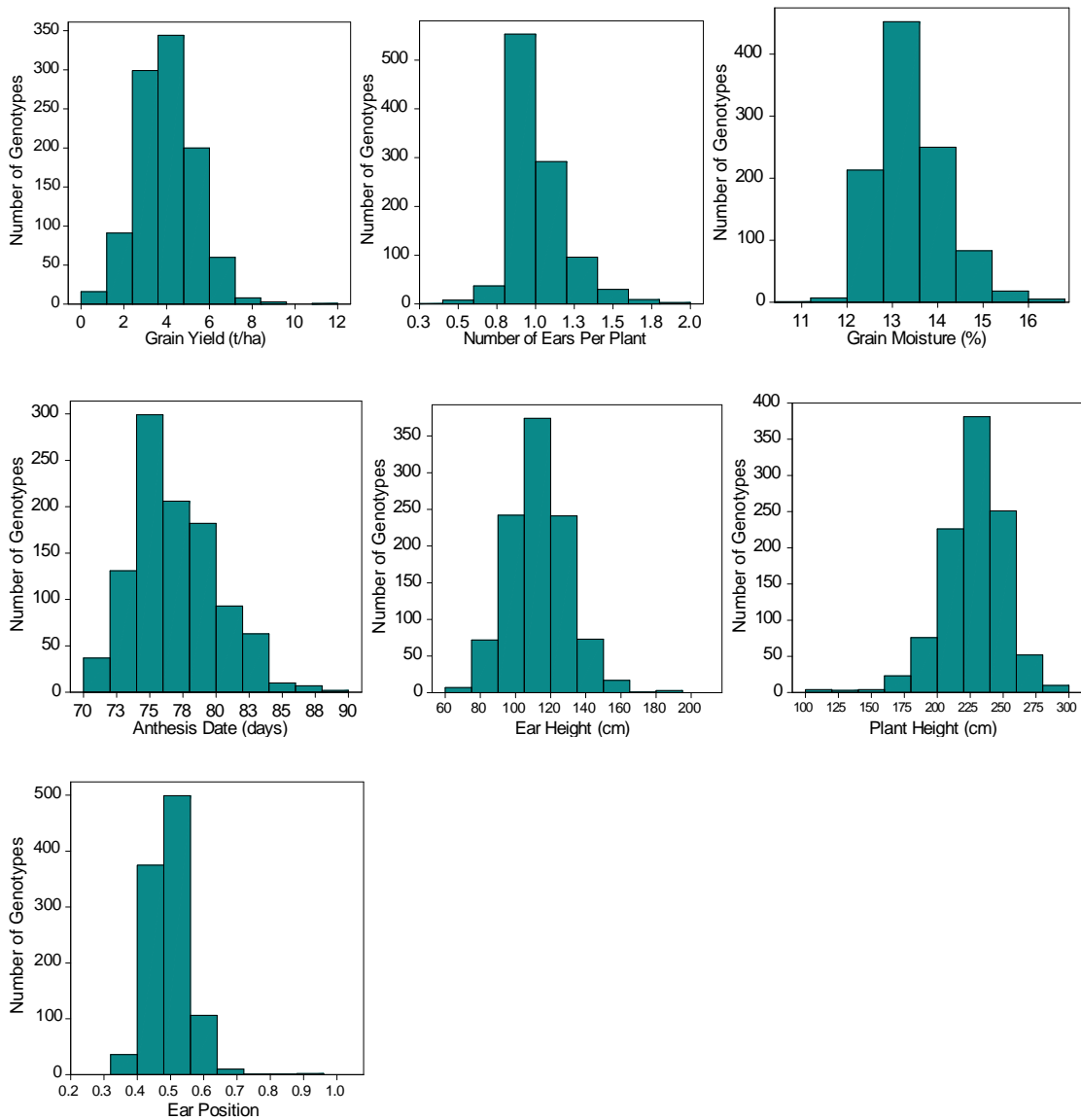


Figure 4-3: Histogram showing variation of the seven traits among 1012 maize hybrids evaluated at Ukulinga (Pietermaritzburg, KZN province, South Africa) in the 2011/2012 season

Cedara results are displayed in Figure 4-4. Grain yield and ear position displayed normal distribution. Number of ears per plant, anthesis date, ear height and plant height exhibited positive skewness; whereas grain moisture exhibited negative skewness. The distribution was continuous for all the traits.

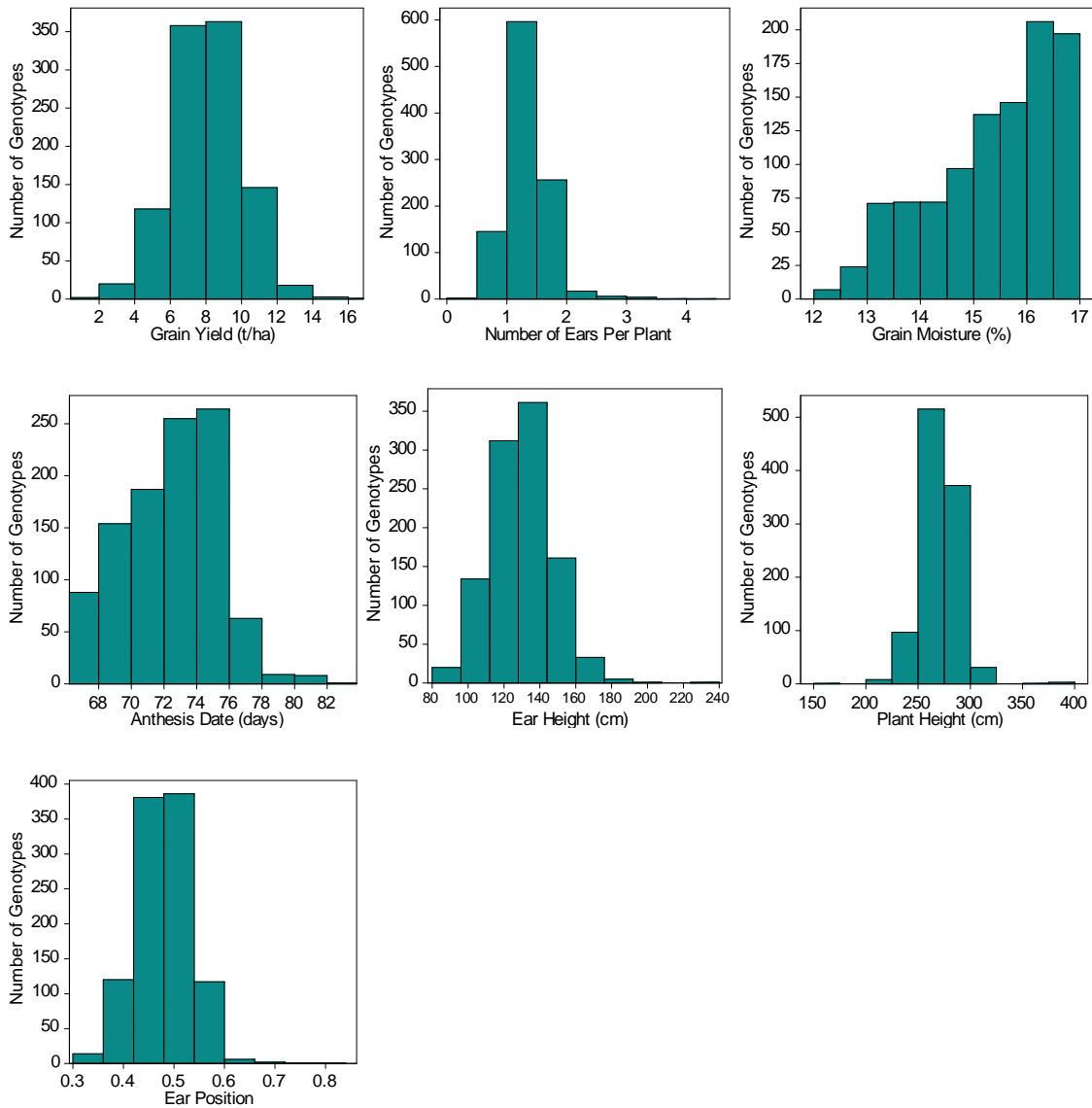


Figure 4-4: Histograms showing variation of seven traits among 1012 maize hybrids evaluated at Cedara (Pietermaritzburg, KZN province, South Africa) in the 2011/2012 season

Dundee results are presented in Figure 4-5. Grain yield, number of ears per plant, ear height and ear position displayed positive skewness; whereas plant height displayed negative skewness. The distribution was continuous for all the traits.

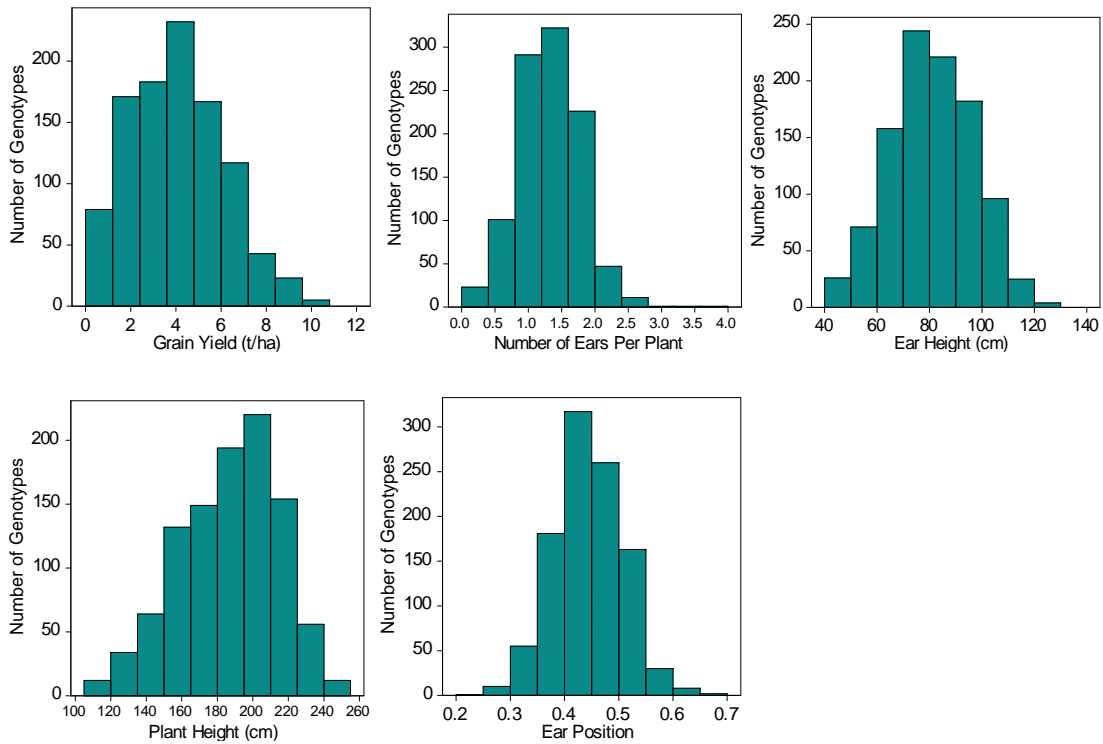


Figure 4-5: Histograms showing variation of five traits among 1012 maize hybrids evaluated at Dundee (KZN province, South Africa) in the 2011/2012 season

Potchefstroom results are displayed in Figure 4-6. Grain yield, number of ears per plant, grain moisture and anthesis date exhibited positive skewness; whereas ear height, plant height and ear position exhibited negative skewness. The distribution was continuous for all the traits except anthesis date and plant height for which distribution was non-continuous.

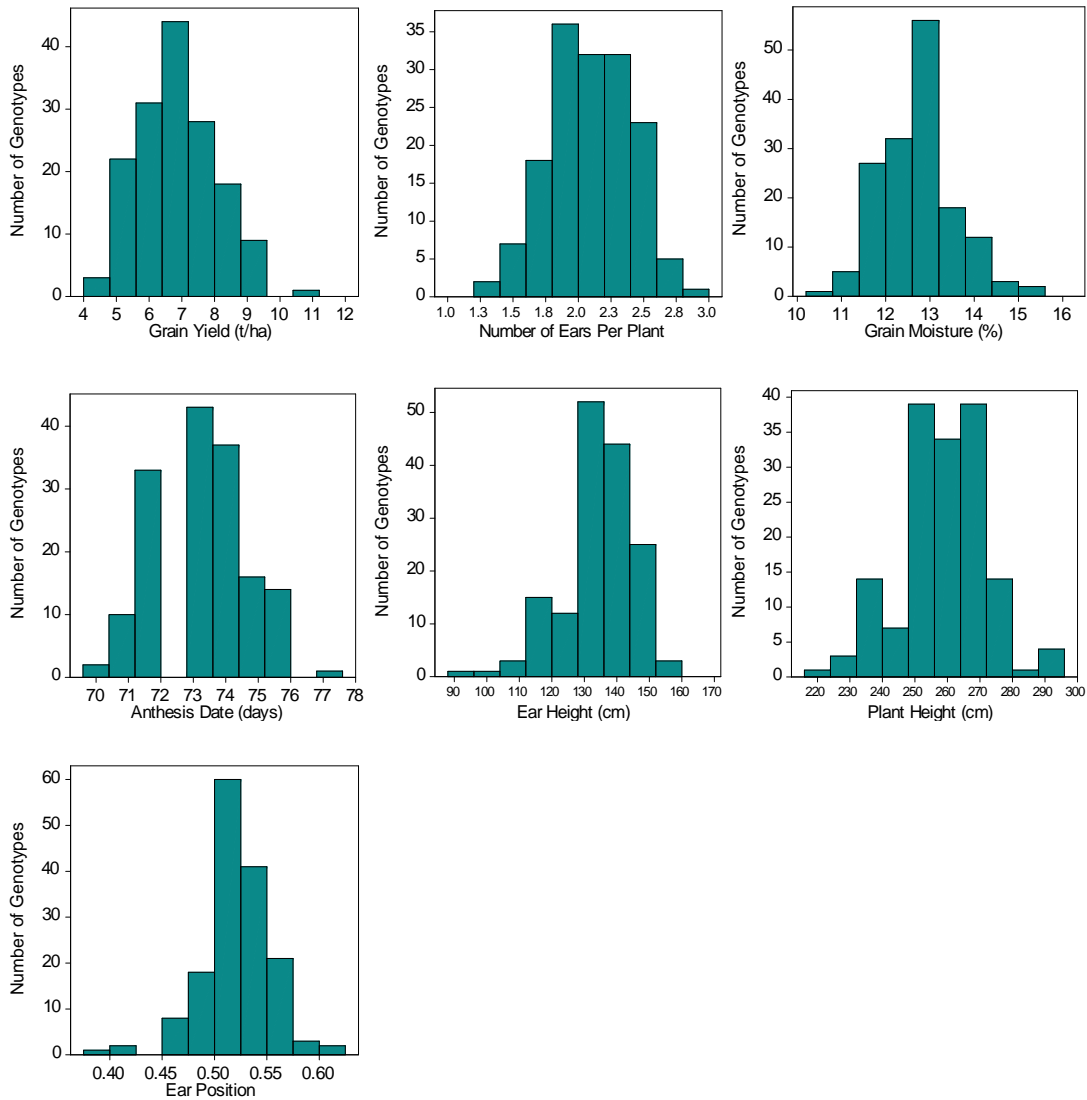


Figure 4-6: Histograms showing variation of seven traits among 132 maize hybrids evaluated at Potchefstroom (North West province, South Africa) in the 2011/2012 season

4.3.3 Phenotypic correlations between grain yield and secondary traits

Only traits which exhibited significant correlation with grain yield will be explained and discussed. Ukulinga results are presented in Table 4-2. Chlorophyll, ear height, ear length, number of ears per plant, grain moisture, kernels per cob, leaf area, plant height and number of primary tassel branches had a significant and positive correlation with grain yield. Whereas ear aspect, ear position and silking date showed a significant and negative correlation with grain yield.

Cedara results are displayed in Table 4-3. Chlorophyll, ear height, ear length, ear position, number of ears per plant, grain moisture, number of kernels per cob, leaf area, number of leaves, plant height and number of primary tassel branches had a significant and positive correlation with grain yield. Whereas anthesis date, ear aspect and silking date exhibited a significant and negative correlation with grain yield.

Potchefstroom results are displayed in Table 4-4. Anthesis date, ear height, number of ears per plant and plant height had significant and positive correlation with grain yield. Whereas ear aspect had a significant and negative correlation with grain yield.

Dundee results are presented in Table 4-5. Ear height, number of ears per plant and plant height had a significant and positive correlation with grain yield.

Table 4-2: Phenotypic correlation coefficients between yield and yield components at Ukulinga research farm

	AD	CHL	EA	EH	EL	EPO	EPP	GM	GT	KPC	LA	NL	PH	SD	TB	Yield
AD	-															
CHL	<u>-0.141</u>	-														
EA	<u>-0.239</u>	<u>-0.195</u>	-													
EH	<u>-0.171</u>	<u>0.126</u>	-0.037	-												
EL	0.013	<u>0.295</u>	<u>-0.369</u>	<u>0.167</u>	-											
EPO	<u>0.171</u>	-0.018	<u>0.149</u>	<u>0.674</u>	-0.019	-										
EPP	<u>-0.068</u>	<u>0.120</u>	-0.058	<u>0.069</u>	-0.020	<u>-0.075</u>	-									
GM	<u>0.400</u>	0.024	<u>-0.446</u>	<u>-0.089</u>	<u>0.151</u>	-0.003	-0.024	-								
GT	<u>-0.379</u>	<u>0.170</u>	<u>0.210</u>	<u>0.072</u>	-0.008	<u>-0.067</u>	<u>0.062</u>	<u>-0.308</u>	-							
KPC	<u>-0.249</u>	<u>0.201</u>	<u>-0.188</u>	<u>0.184</u>	<u>0.232</u>	<u>-0.130</u>	<u>0.113</u>	<u>-0.074</u>	<u>0.138</u>	-						
LA	<u>-0.309</u>	<u>0.303</u>	-0.068	<u>0.357</u>	<u>0.271</u>	0.035	<u>0.070</u>	<u>-0.085</u>	0.025	<u>0.264</u>	-					
NL	<u>0.279</u>	<u>0.092</u>	<u>-0.124</u>	<u>0.121</u>	<u>-0.118</u>	<u>0.229</u>	<u>0.102</u>	<u>0.215</u>	-0.074	-0.055	<u>-0.115</u>	-				
PH	<u>-0.405</u>	<u>0.182</u>	<u>-0.191</u>	<u>0.632</u>	<u>0.237</u>	<u>-0.137</u>	<u>0.158</u>	<u>-0.122</u>	<u>0.160</u>	<u>0.393</u>	<u>0.454</u>	<u>-0.082</u>	-			
SD	<u>0.985</u>	<u>-0.147</u>	<u>-0.235</u>	<u>-0.177</u>	0.028	<u>0.167</u>	-0.096	<u>0.402</u>	<u>-0.383</u>	<u>-0.250</u>	<u>-0.318</u>	<u>0.266</u>	<u>-0.410</u>	-		
TB	<u>-0.158</u>	<u>0.142</u>	0.038	<u>0.079</u>	<u>0.130</u>	<u>0.067</u>	0.031	-0.002	0.046	0.036	<u>0.213</u>	<u>0.074</u>	0.035	<u>-0.155</u>	-	
Yield	-0.053	<u>0.380</u>	<u>-0.581</u>	<u>0.158</u>	<u>0.431</u>	<u>-0.174</u>	<u>0.275</u>	<u>0.251</u>	0.060	<u>0.326</u>	<u>0.257</u>	0.060	<u>0.388</u>	<u>-0.063</u>	<u>0.077</u>	-

Underlined, bold and underlined = significant at $P \leq 0.05$ and $P \leq 0.001$, respectively. AD = anthesis date. CHL = chlorophyll content. EA = ear aspect score (1-5). EH = ear height. EL = ear length. EPO = ear position. EPP = number of ears per plant. GM = grain moisture content (%). GT = grain texture score (1-5). KPC = number of kernels per cob. LA = ear leaf area. NL = number of leaves per plant. PH = plant height. SD = silking date. TB = number of primary tassel branches. Yield = grain yield.

Table 4-3: Phenotypic correlation coefficients between yield and yield components at Cedara research station

	AD	CHL	EA	EH	EL	EPO	EPP	GLS	GM	GT	KPC	LA	NL	PH	PLS	SD	TB	Yield	
AD	-																		
CHL	<u>-0.131</u>	-																	
EA	0.014	<u>-0.097</u>	-																
EH	<u>0.205</u>	0.050	<u>-0.103</u>	-															
EL	<u>0.083</u>	<u>0.153</u>	<u>-0.213</u>	<u>0.081</u>	-														
EPO	<u>0.194</u>	0.038	-0.033	<u>0.862</u>	0.041	-													
EPP	<u>-0.064</u>	<u>0.065</u>	<u>-0.072</u>	-0.015	<u>-0.155</u>	0.007	-												
GLS	<u>-0.134</u>	-0.037	0.042	<u>-0.139</u>	0.026	<u>-0.163</u>	0.026	-											
GM	<u>0.196</u>	0.031	<u>-0.221</u>	<u>0.075</u>	<u>0.140</u>	0.047	<u>-0.099</u>	-0.057	-										
GT	<u>-0.203</u>	<u>0.087</u>	<u>0.151</u>	-0.063	0.061	<u>-0.073</u>	-0.023	-0.017	<u>-0.206</u>	-									
KPC	-0.050	0.039	<u>-0.152</u>	<u>0.080</u>	<u>0.103</u>	0.009	0.002	0.028	0.036	<u>0.097</u>	-								
LA	<u>0.073</u>	<u>0.072</u>	<u>-0.245</u>	<u>0.083</u>	<u>0.128</u>	0.018	<u>0.106</u>	0.029	<u>0.080</u>	0.050	<u>0.134</u>	-							
NL	<u>0.103</u>	0.038	<u>-0.076</u>	<u>0.318</u>	<u>-0.134</u>	<u>0.271</u>	<u>0.069</u>	<u>-0.122</u>	<u>0.116</u>	<u>-0.117</u>	0.031	<u>-0.064</u>	-						
PH	<u>0.065</u>	0.031	<u>-0.145</u>	<u>0.511</u>	<u>0.080</u>	0.014	-0.040	0.005	<u>0.062</u>	-0.006	<u>0.144</u>	<u>0.118</u>	<u>0.177</u>	-					
PLS	<u>-0.134</u>	-0.012	<u>0.090</u>	-0.024	<u>-0.127</u>	-0.016	<u>0.087</u>	<u>-0.077</u>	<u>-0.084</u>	<u>0.098</u>	0.038	-0.060	-0.014	-0.025	-				
SD	<u>0.990</u>	<u>-0.132</u>	0.012	<u>0.214</u>	0.085	<u>0.205</u>	-0.066	<u>-0.134</u>	<u>0.192</u>	<u>-0.199</u>	-0.048	0.074	<u>0.100</u>	<u>0.065</u>	<u>-0.127</u>	-			
TB	-0.050	0.061	<u>-0.077</u>	<u>0.076</u>	<u>0.143</u>	<u>0.137</u>	<u>-0.122</u>	<u>-0.073</u>	<u>0.099</u>	0.056	-0.053	-0.055	<u>0.154</u>	<u>-0.088</u>	<u>-0.132</u>	-0.040	-		
Yield	<u>-0.083</u>	<u>0.145</u>	<u>-0.453</u>	<u>0.257</u>	<u>0.213</u>	<u>0.137</u>	<u>0.209</u>	-0.044	<u>0.169</u>	-0.056	<u>0.131</u>	<u>0.141</u>	<u>0.188</u>	<u>0.272</u>	-0.055	<u>-0.082</u>	<u>0.094</u>	-	

Underlined, bold and underlined = significant at $p \leq 0.05$ and $p \leq 0.001$, respectively. AD = anthesis date. CHL = chlorophyll content. EA = ear aspect score (1-5). EH = ear height. EL = ear length. EPO = ear position. EPP = number of ears per plant. GLS = grey leaf spot disease. GM = grain moisture content. GT = grain texture score (1-5). KPC = kernels per cob. LA = ear leaf area. NL = number of leaves per plant. PH = plant height. PLS = phaeosphaeria leaf spot disease. SD = silking date. TB = number of primary tassel branches. Yield = grain yield.

Table 4-4: Phenotypic correlation coefficients between yield and yield components at ARC-Potchefstroom research station

	AD	EA	EH	EPO	EPP	GM	PH	SD	Yield
AD	-								
EA	<u>-0.236</u>	-							
EH	<u>0.328</u>	<u>-0.360</u>	-						
EPO	<u>0.220</u>	<u>-0.162</u>	<u>0.765</u>	-					
EPP	<u>0.122</u>	<u>-0.163</u>	0.069	-0.023	-				
GM	0.127	<u>-0.356</u>	0.085	0.041	0.143	-			
PH	<u>0.229</u>	<u>-0.342</u>	<u>0.554</u>	-0.111	0.143	0.067	-		
SD	<u>0.832</u>	<u>-0.335</u>	<u>0.406</u>	<u>0.276</u>	-0.006	0.156	<u>0.277</u>	-	
Yield	<u>0.202</u>	<u>-0.444</u>	<u>0.210</u>	0.026	<u>0.438</u>	0.110	<u>0.290</u>	0.157	-

Underlined, bold and underlined = significant at $p \leq 0.05$ and $p \leq 0.001$, respectively. AD = anthesis date. EA = ear aspect score (1-5). EH = ear height. EPO = ear position. EPP = number of ears per plant. GM = grain moisture content. PH = plant height. SD = silking date. Yield = grain yield.

Table 4-5: Phenotypic correlation coefficients between yield and yield components at Dundee research station

	EH	EPO	EPP	PH	Yield
EH	-				
EPO	<u>0.614</u>	-			
EPP	<u>0.389</u>	-0.006	-		
PH	<u>0.702</u>	<u>-0.118</u>	<u>0.506</u>	-	
Yield	<u>0.458</u>	-0.023	<u>0.547</u>	<u>0.610</u>	-

Bold and underlined = significant at $p \leq 0.001$. EH = ear height. EPO = ear position. EPP = number of ears per plant. PH = plant height. Yield = grain yield.

4.3.4 Path coefficient analysis

Ukulinga results are presented in Table 4-6. At Ukulinga, ear length had the highest direct effect on grain yield, followed by grain moisture and plant height, anthesis date, chlorophyll, number of ears per plant, number of kernels per cob, number of leaves per plant and leaf area. Anthesis date had the highest indirect effect on grain yield through silking date, followed by plant height through leaf area.

Cedara results are displayed in Table 4-7. At Cedara, number of ears per plant had the highest direct effect on grain yield, followed by plant height, ear length, moisture content,

number of leaves per plant, ear position, number of primary tassel branches and leaf area, number of kernels per cob and chlorophyll. Anthesis date, silking date, grey leaf spot disease scores and phaeosphaeria leaf spot disease scores had negative direct effect on grain yield. All the traits exhibited little indirect effects on grain yield. However, anthesis date had the highly negative indirect effect on grain yield through silking date. Potchefstroom results are shown in Table 4-8.

At Potchefstroom, number of ears per plant had the highest direct effect on grain yield, followed by plant height, anthesis date, silking date and ear position, and grain moisture content. Traits displayed little indirect effects on grain yield. However, ear position had high indirect effect on grain yield through grain moisture content; anthesis date and plant height also had high indirect effects on grain yield through silking date.

At Dundee, plant height had the highest direct effect on grain yield, followed by ear position. Plant height had a highly negative indirect effect on grain yield through ear position; whereas ear position had a slightly negative indirect effect on grain yield through plant height.

Table 4-6: Direct (underlined and bold) and indirect effects of different traits in maize at Ukulinga research farm

Grain yield component	EPP	GM	AD	SD	PH	EPO	NL	TB	CHL	LA	EL	KPC	The total correlation to grain yield
EPP	<u>0.19</u>	-0.01	-0.01	0.02	0.04	0.01	0.00	0.00	0.02	0.00	-0.01	0.01	0.28
GM	0.00	<u>0.23</u>	0.09	-0.08	-0.03	0.00	0.01	0.00	0.00	0.00	0.04	-0.01	0.25
AD	-0.01	0.09	<u>0.22</u>	-0.19	-0.09	-0.02	0.01	0.00	-0.03	0.00	0.00	-0.03	-0.05
SD	-0.02	0.09	0.21	<u>-0.19</u>	-0.10	-0.02	0.01	0.00	-0.03	0.00	0.01	-0.03	-0.06
PH	0.03	-0.03	-0.09	0.08	<u>0.23</u>	0.02	0.00	0.00	0.04	0.00	0.06	0.05	0.39
EPO	-0.01	0.00	0.04	-0.03	-0.03	<u>-0.12</u>	0.01	0.00	0.00	0.00	0.00	-0.02	-0.17
NL	0.02	0.05	0.06	-0.05	-0.02	-0.03	<u>0.05</u>	0.00	0.02	0.00	-0.03	-0.01	0.06
TB	0.01	0.00	-0.03	0.03	0.01	-0.01	0.00	<u>0.00</u>	0.03	0.00	0.03	0.00	0.08
CHL	0.02	0.01	-0.03	0.03	0.04	0.00	0.00	0.00	<u>0.20</u>	0.00	0.08	0.02	0.38
LA	0.01	-0.02	-0.07	0.06	0.11	0.00	-0.01	0.00	0.06	<u>0.01</u>	0.07	0.03	0.26
EL	0.00	0.03	0.00	-0.01	0.06	0.00	-0.01	0.00	0.06	0.00	<u>0.26</u>	0.03	0.43
KPC	0.02	-0.02	-0.05	0.05	0.09	0.02	0.00	0.00	0.04	0.00	0.06	<u>0.12</u>	0.33

EPP = number of ears per plant. GM = grain moisture content. AD = anthesis date. SD = silking date. PH = plant height. EPO = ear position. NL = number of leaves per plant. TB = number of primary tassel branches. CHL = chlorophyll content. LA = ear leaf area. EL = ear length. KPC = number of kernels per cob. Yield = grain yield.

Table 4-7: Direct (underlined and bold) and indirect effects of different traits in maize at Cedara research station

Grain yield component	EPP	GM	AD	SD	PH	EPO	NL	TB	CHL	LA	EL	KPC	GLS	PLS	Total correlation to grain yield
EPP	<u>0.25</u>	-0.01	0.01	0.00	-0.01	0.00	0.01	-0.01	0.00	0.01	-0.03	0.00	0.00	0.00	0.21
GM	-0.02	<u>0.14</u>	-0.02	-0.01	0.01	0.00	0.01	0.01	0.00	0.00	0.03	0.00	0.00	0.00	0.17
AD	-0.02	0.03	<u>-0.13</u>	-0.04	0.02	0.02	0.01	0.00	-0.01	0.00	0.02	0.00	0.01	0.01	-0.08
SD	-0.02	0.03	-0.12	<u>-0.04</u>	0.02	0.02	0.01	0.00	-0.01	0.00	0.02	0.00	0.01	0.01	-0.08
PH	-0.01	0.01	-0.01	0.00	<u>0.23</u>	0.00	0.02	0.00	0.00	0.01	0.02	0.01	0.00	0.00	0.27
EPO	0.00	0.01	-0.02	-0.01	0.00	<u>0.10</u>	0.03	0.01	0.00	0.00	0.01	0.00	0.01	0.00	0.14
NL	0.02	0.02	-0.01	0.00	0.04	0.03	<u>0.12</u>	0.01	0.00	0.00	-0.03	0.00	0.01	0.00	0.19
TB	-0.03	0.01	0.01	0.00	-0.02	0.01	0.02	<u>0.06</u>	0.00	0.00	0.03	0.00	0.00	0.01	0.09
CHL	0.02	0.00	0.02	0.01	0.01	0.00	0.00	0.00	<u>0.04</u>	0.00	0.03	0.00	0.00	0.00	0.14
LA	0.03	0.01	-0.01	0.00	0.03	0.00	-0.01	0.00	0.00	<u>0.06</u>	0.03	0.01	0.00	0.00	0.14
EL	-0.04	0.02	-0.01	0.00	0.02	0.00	-0.02	0.01	0.01	0.01	<u>0.20</u>	0.01	0.00	0.01	0.21
KPC	0.00	0.01	0.01	0.00	0.03	0.00	0.00	0.00	0.00	0.01	0.02	<u>0.05</u>	0.00	0.00	0.13
GLS	0.01	-0.01	0.02	0.01	0.00	-0.02	-0.01	0.00	0.00	0.00	0.01	0.00	<u>-0.04</u>	0.00	-0.04
PLS	0.02	-0.01	0.02	0.00	-0.01	0.00	0.00	-0.01	0.00	0.00	-0.03	0.00	0.00	<u>-0.05</u>	-0.06

EPP = number of ears per plant. GM = grain moisture content. AD = anthesis date. SD = silking date. PH = plant height. EPO = ear position. NL = number of leaves. TB = number of primary tassel branches. CHL = chlorophyll content. LA = ear leaf area. EL = ear length. KPC = number of kernels per cob. GLS = grey leaf spot. PLS = phaeosphaeria leaf spot.

Table 4-8: Direct (underlined and bold) and indirect effects of different traits in maize at ARC-Potchefstroom research station

Grain yield component	EPP	GM	AD	SD	PH	EPO	Total correlation to grain yield
EPP	<u>0.40</u>	0.00	0.01	0.00	0.03	0.00	0.44
GM	0.06	<u>0.02</u>	0.01	0.00	0.01	0.00	0.11
AD	0.05	0.00	<u>0.07</u>	0.02	0.05	0.01	0.20
SD	0.00	0.00	0.06	<u>0.03</u>	0.06	0.01	0.16
PH	0.06	0.00	0.02	0.01	<u>0.21</u>	0.00	0.29
EPO	-0.01	0.00	0.02	0.01	-0.02	<u>0.03</u>	0.03

EPP = number of ears per plant. GM = grain moisture content. AD = anthesis date. SD = silking date. PH = plant height. EPO = ear position.

4.4 DISCUSSION

4.4.1 Genetic variation, heritability and genetic advance

The weather data showed that on average Potchefstroom had the highest rainfall throughout the growing season followed by Ukulinga, Cedara and Dundee. The weather data also showed that Potchefstroom had the highest average temperature throughout the growing season followed by Ukulinga, Dundee and Cedara. The results further indicated that even within the same site the environmental conditions were highly variable at different growth stages. Therefore, the environmental conditions across these sites were different throughout the growing season. Hence, these results demonstrate that the differences in the phenotypic and genotypic performance of the plants were expected. Environmental conditions largely contributed to the differences in genotypic performance.

The results are in accordance with Mitrovic et al. (2012) who reported that the highest percentage of variation in their study was explained by environment. Beyene et al. (2011) also reported the environment main effect to be the most important source of variation for all the studied traits except for anthesis to silking interval. Gissa (2008) reported that PCV and GCV were the highest for grain yield and anthesis-silking interval. Bello (2012) reported that there were significant differences among the genotypes for measured characters; PCV was slightly higher than GCV for all the characters suggesting the presence of environmental influence to some extent in the expression of these characters. Nadagoud (2008) reported

that PCV was comparatively higher than that of the GCV (suggesting the influence of environmental factor on all characters); the GCV and PCV revealed moderate to high variability for majority of the characters.

Of all the studied sites, grain yield was highly heritable at Potchefstroom, followed by Cedara, Ukulinga and Dundee. The high heritability of grain yield at Potchefstroom can be attributed to the fact that rainfall and temperature were adequate at flowering growth stage where most of grain yield is identified. Potchefstroom is the only location where genetic variation was higher than environmental variation for grain yield; hence, heritability of grain yield was the highest at Potchefstroom. At other three locations, heritability of grain yield might have been low because environmental variation contributed more than genetic variation towards grain yield. Especially in Dundee, where grain yield was retarded by a number of external environmental factors pre and post flowering which significantly lowered plant performance, hence, lower heritability of this trait. Cedara had the highest genetic improvement in yield followed by Potchefstroom, Dundee and Ukulinga, respectively. However, in terms of genetic advance percentage with respect to the mean (GAM), Dundee had the highest followed by Potchefstroom, Ukulinga and Cedara, respectively.

The number of ears per plant was highly heritable at Potchefstroom, followed by Ukulinga, Dundee and Cedara. The number of ears per plant is the important determinant of grain yield; hence, in most cases when grain yield is high it is mainly due to the number of ears per plant. However, the environmental conditions at Potchefstroom were favourable for good plant performance, especially at flowering where grain yield is mostly determined, even though they were not entirely favourable at later growth stages when winds and storms were experienced. Hence, the heritability of number of ears per plant was average ($\approx 50\%$) due to equal effect of genetic and environmental variation. The results show that Potchefstroom had a highest genetic improvement in number of ears per plant followed by Dundee, Cedara and Ukulinga. However, in terms of GAM Dundee had the highest followed by Ukulinga, Cedara and Potchefstroom, respectively. Anthesis date was highly heritable at Ukulinga, followed by Cedara and Potchefstroom. All these locations had heritability above

50%. This was also due to the predominance of genetic variation over environmental variation. The results show that Ukulinga had the highest genetic improvement in anthesis date, followed by Cedara, then Potchefstroom. The similar trend was also observable for GAM. Therefore, Ukulinga obtained more increase in the lateness of anthesis in genotypes; hence, it should be used in the breeding program if the objective of the study is to explore the lateness in anthesis date. These results thus show the possible reason for lower yield experienced at Ukulinga, because usually the late flowering genotypes are low in yield, because it is an indication of lack of adaptation when genotypes with a tropical genome are grown in South Africa. Ear height was highly heritable at Potchefstroom, followed by Cedara, Ukulinga and Dundee. Because at Potchefstroom there was the predominance of genetic variation over environmental variation. The results show that Potchefstroom had highest genetic improvement in ear height, followed by Cedara, Ukulinga and Dundee, respectively. However, in terms of GAM Potchefstroom had the highest followed by Ukulinga, Cedara and Dundee, respectively. Hence, the environmental conditions in Potchefstroom favour the increase in maize plant ear heights, whereas Dundee environmental conditions result into a least improvement in ear heights. Plant height was highly heritable at Potchefstroom, followed by Cedara, Dundee and Ukulinga. This is mainly because plant height at Potchefstroom and Cedara was largely controlled by genetic variation rather than environmental variation. Cedara had the highest genetic improvement in plant height, followed by Potchefstroom, Dundee and Ukulinga. But in terms of GAM Dundee was leading, followed by Cedara, Potchefstroom and Ukulinga, respectively. Therefore, the growing of plants in Cedara and Potchefstroom can result into a dramatic increase in the expression of genes for plant height increment which can thus make the plants prone to lodging. Ear position was highly heritable at Potchefstroom, followed by Ukulinga, Cedara and Dundee. This is mainly because at Potchefstroom and Ukulinga ear position was largely controlled by genetic variation instead of environmental variation as was observed at Cedara and Dundee. Ukulinga had the highest genetic improvement in ear position, followed by Potchefstroom, Cedara and Dundee. The similar trend was also witnessed for GAM.

Several authors reported low, medium and high estimates of heritability in different plant characters in different environments working with different hybrids. Mahmood et al. (2004) reported highest heritability estimates for grain yield (0.993) and plant height (0.990). Mahmood et al. (2004) also reported values of genetic advance ranging between 43.80 for grain yield to 1.33 for number of kernel rows per ear. Gissa (2008) estimated heritability to be the highest for anthesis date and grain weight. Bello (2012) reported high magnitude of heritability coupled with genetic advance for all the traits except anthesis date. Nadagoud (2008) reported heritability estimates to range from 64.93% (number of kernel rows per cob) to 96.80% (grain yield); anthesis date (89.27%), silking date (88.57%), plant height (93.53%), ear height (92.77%), ear length (85.97%), number of kernels per row (72.80%) and grain yield (96.80%). Genetic advance ranged from 6.75% to 44.57%; the GA for grain yield (44.57%), ear length (20.75%), plant height (26.40%) and ear height (28.64%) were high; whereas number of kernel rows per ear (15.38%) and number of kernels per row (19.46%) showed moderate genetic advance; while anthesis date and silking date showed low magnitude of GA (Nadagoud, 2008).

4.4.2 Variation of hybrids for grain yield and associated traits

At Ukulinga grain yield, number of ears per plant, grain moisture, anthesis date and ear position all exhibited positive skewness. But ear height showed normal distribution; whereas plant height exhibited negative skewness. Grain yield ranged from 0 to 9 t ha⁻¹; this can be attributed to environmental conditions at Ukulinga during the critical growth stage (flowering), where the rainfall was very low and the temperatures were very high above all the other three sites at this growth stage; hence, there are very few genotypes which were able to produce high yields in those kinds of environmental conditions. The distribution was continuous for all the traits, which demonstrate that all the traits were controlled by many genes; which imply that these traits cannot be successfully selected in the breeding program. The results are in accordance with Holland (2007) who reported that genetic variation for complex traits, such as yield potential in elite maize populations is controlled by many genetic factors, each with relatively small effects. Many genotypes also produced fewer number of ears per plant, low grain moisture, low anthesis date and ear position. Many genotypes produced the lower number of ears per plant probably because the growth

assimilates were more focussed on plant growth rather than towards an increase in number of ears per plant. The taller the plants the greater the chances for higher ear position, as was experienced. Many genotypes also displayed lower grain moisture at harvest which means they were drying very fast, which is one of the prerequisite for earliness; hence, many genotypes showed fewer days to anthesis.

At Cedara, grain yield and ear position displayed normal distribution. However, number of ears per plant, anthesis date, ear height and plant height exhibited positive skewness; whereas grain moisture exhibited negative skewness. The majority of the genotypes produced grain yield between 8 and 10 t ha⁻¹, and the highest observed grain yield was around 16 t ha⁻¹. The results show that Cedara was the high yielding environment, but the rainfall at Cedara was the lowest at the critical growth stage (flowering), however, the temperatures were also the lowest. Therefore, the majority of the genotypes at Cedara were able to produce high yields even in the absence of adequate rainfall at critical growth stages. The distribution was continuous for all the traits which thus emphasize that all the traits were controlled by many genes. The results are in similarity with Holland (2007) who reported that genetic variation for complex traits, such as yield potential in elite maize populations is controlled by many genetic factors, each with relatively small effects.

The majority of the genotypes at Cedara also produced a desired ear position (0.50), hence, this will also form part of the selection basis of the exceptional genotypes. The results also showed that ear position was not altered by variations of environmental conditions in Cedara; hence, it was just normally distributed across all genotypes. However, many plants displayed low number of ears per plant, fewer days to anthesis, lower ear heights and plant heights. The results for number of ears per plant show that the higher grain yield which was observed at Cedara was not mainly due to number of ears per plant, therefore the yield in Cedara can be attributed to some yield components such as number of kernels per ear and ear length. Fewer days to anthesis indicate that majority of plants in Cedara flowered very early, which is therefore one of the possible reasons for higher yields in Cedara despite lower rainfall and temperature conditions experienced. Lower ear and plant heights were observed for the majority of plants; hence, this might have been helpful during the course

of heavy winds and storms to culminate lodging, and thus higher grain yields were observed in Cedara. These lower ear and plant heights were possibly due to the insufficiency of rainfall to support further plant growth. Frova et al. (1999) also reported a normal frequency distribution of the genotypes across well watered and stressed environments.

At Dundee, grain yield, number of ears per plant, ear height and ear position displayed positive skewness; whereas plant height displayed negative skewness. The grain yield at Dundee ranged between 0 and 11 t ha⁻¹, where majority of the genotypes produced 4 t ha⁻¹. The results demonstrate that majority of the genotypes produced lower yields. The results are contradicting the environmental conditions at Dundee, because this environment had the adequate rainfall at critical growth stage (flowering) and also the temperatures were reasonably adequate for higher yields. Also, ear height and ear position at Dundee displayed negative skewness, which suggests that the majority of genotypes produced below average ear height and ear position; hence, the chances of yield loss through lodging are very low, because based on the results the genotypes at Dundee were very short probably shorter than all the other studied environments. The distribution was continuous for all the traits which explicate that all the traits were controlled by many genes. Hence, these traits cannot be easily modified genetically due to the presence of low gene intensity. The results are in accordance with Holland (2007) who reported that genetic variation for complex traits, such as yield potential in elite maize populations is controlled by many genetic factors, each with relatively small effects.

At Potchefstroom, grain yield, number of ears per plant, grain moisture and anthesis date exhibited positive skewness; whereas ear height, plant height and ear position exhibited negative skewness. Grain yield at Potchefstroom ranged from 4 to 11 t ha⁻¹, but the results show that the majority of the genotypes produced below average grain yields. However, the results also show that ear height, plant height and ear position, both displayed positive skewness, meaning that the majority of the genotypes at Potchefstroom had above average ear height, plant height and ear position and these were very high. At the same time the weather data, showed that the rainfall during the critical growth stage was reasonably high and also the temperature conditions were adequate for successful fertilization and general

plant growth. Therefore, this pattern of below average grain yield at Potchefstroom may be attributed to other environmental conditions such as wind and storms that occurred prior to harvesting, which possibly resulted in subsequent loss of grain yield through lodging, since the ear height, plant height and ear position were very high and hence the plants were prone to such conditions. The distribution was continuous for all the traits except anthesis date and plant height which elucidate that the other traits were controlled by poly genes whereas anthesis date and plant height were controlled by fewer genes. Hence, the presence of many genes controlling these traits can thus complicate the manipulation of these traits except anthesis date and plant height. Such conditions prior to harvesting possibly affected other grain yield components such as number of ears per plant as the results display that the majority of the genotypes showed lower number of ears per plant. The results also show that the majority of genotypes exhibited lower grain moisture and days to anthesis, which demonstrate that the genotypes were early and they were thus not prone to late disease attack.

4.4.3 Phenotypic correlations between grain yield and secondary traits

Chlorophyll, ear height, ear length, number of ears per plant, grain moisture, kernels per cob, leaf area, plant height and number of primary tassel branches had a significant and positive correlation with grain yield. These results show that an increase in these traits resulted to an increase in grain yield. Hence, these traits can be manipulated for yield increment. Whereas ear aspect, ear position (Ukulinga), silking date and anthesis date (Cedara) showed a significant and negative correlation with grain yield. The results show that ear position need to be low for grain yield to increase, and also the earlier the genotypes at Ukulinga the higher the grain yield. Therefore, all these traits can be manipulated accordingly for grain yield increment. However, at Potchefstroom the unexpected happened where anthesis date had significant and positive correlation with grain yield. These results show that an increase in anthesis date at Potchefstroom resulted to a significant increase in grain yield. However, there is still a need to further determine the relationship between grain yield and anthesis date at Potchefstroom. There is a need to study the behaviour of secondary traits towards each other. Hence, the correlation between the traits will be elaborated. There are other studies which have been reported by different

authors on the relationship of these traits with grain yield under different environmental conditions. Selvaraj and Nagarajan (2011), Tan et al. (2006) and Pradeep and Satyanarayana (2001) reported that characters like plant height, ear height, ear length and number of grains per ear showed significant positive association with grain yield. Gholamin and Khayatnezhad (2011) and Zaidi et al. (2008) reported significant and positive correlation between chlorophyll content and grain yield. Hefny (2011) reported that anthesis date and silking date associated negatively with grain yield. These results are in accordance with the current study, and they have implications for indirect selection where grain yield can be indirectly increased through breeding selection for these traits.

4.4.4 Phenotypic correlations among secondary traits

The results demonstrate the relationship between the secondary and adaptive traits. These associations' studies are useful because they show which traits can be increased concurrently, for instance if two traits have a significant and positive correlation this implies that if you increase one trait there is no need to increase another individually because they complement each other. Some traits displayed the similar trend across all the sites which showed that those traits which were significantly correlated with each other repeatedly across these sites were reliable for indirect selections. Therefore, they can be manipulated in the future breeding programmes. But those which displayed varying trends across the sites show that their relationship towards each other is affected by environmental conditions. The results also showed that there are traits which had a suppressing relationship toward each other across the sites. These results alert the breeders that if they intend increasing a certain trait which traits are being compromised. There are also certain traits which strictly had no significant correlation across the sites. There are several authors who have reported on the relationship between secondary traits using different hybrids and studying them in environments different from the current. Mohammad et al. (2008) reported that plant height had highly significant association with ear height and anthesis date with silking date; these results are in accordance with the current study since the similar trends were observed across all the sites for these traits. Zaidi et al. (2008) reported a significant correlation between grain moisture and chlorophyll content, which is in contrast with the current study because there was no significant correlation between these

traits. Iqbal et al. (2011) reported a significant and negative correlation between silking date and ear length; these results are in contrast with the current study since there was no significant relationship observed between these traits.

4.4.5 Path coefficient analysis

The results showed that plant height had the generally high direct effect on grain yield across all the sites, especially at Dundee. These results show that plant height can be used as the main selection criteria for grain yield and it is least affected by indirect factors. The similar trend was also observed for number of ears per plant, especially at Cedara and Potchefstroom. However, at Ukulinga, ear length had the highest direct effect on grain yield and can be used as a primary selection criterion when breeding for grain yield at Ukulinga and similar environments since its positive direct effect on grain yield is least affected by indirect factors or traits. Number of primary tassel branches had no direct effect on grain yield however, they had an overall positive correlation with grain yield, therefore primary tassel branches affect yield indirectly positively and negatively through other traits. Silking date and ear position had negative direct effects on grain yield; this calls for minimisation of these traits for grain yield improvement. Anthesis date had the highest indirect effect on grain yield through silking date, followed by plant height through leaf area. At Cedara anthesis date, silking date, grey leaf spot and phaeosphaeria leaf spot had negative direct effect on grain yield; these traits had the highest directly negative effect on grain yield, and the breeder should always select against these traits since they largely reduce grain yield. All the traits exhibited little indirect effects on grain yield. However, anthesis date had the highly negative indirect effect on grain yield through silking date. At Potchefstroom, the traits displayed little indirect effects on grain yield. However, ear position, anthesis date and plant height had high indirect effects on grain yield.

There are authors who have reported on the direct and indirect effects of traits on grain yield using genotypes and environments different from the current. Arun and Singh (2004) reported that silking date had the maximum positive direct effect on grain yield; whereas anthesis date had maximum negative effect on grain yield. These results are in contrast with the current study except at Potchefstroom where the similar trend was observed. Selvaraj

and Nagarajan (2011) reported that plant height recorded negative direct effect on grain yield; these results are in contrast with the current study. However, Swarnalatha and Shaik (2001) reported that plant height, silking date, ear length and number of kernels per ear positively influenced the yield directly and also indirectly through several yield components; and these results are in accordance with the current study. Singh et al. (2003) reported that ear length had the maximum direct effect on grain yield; these results are in accordance with the results observed at Cedara. Saidaiah et al. (2008) reported that positive indirect effect on yield was by plant height via plant height, ear height, number of leaves per plant above ear, chlorophyll content, flag area and ear length; these results are in accordance with the current study. Viola et al. (2003) revealed that early silking date, greater plant height, ear length, ear height and number of ears per plant directly contributed to increased ear yield, which is in accordance with the current study.

4.5 CONCLUSION

The objectives of the study were to determine genetic variation among adaptive traits in hybrids. The findings provided sufficient evidence that environmental variations largely contributed to hybrid performance rather than genotypic variations in both grain yield and its adaptive traits because the four sites were very different and represented different environment domains. However, the study confirmed that there was high genetic variation among the hybrids for different traits which increased the genetic base for selection of desired hybrids for future breeding programmes. Grain yield was highly heritable at all sites ranging between 27 and 70% indicating that direct selection for yield would be effective in all environments that were represented by these sites. The distribution of hybrids displayed differing patterns of variations for different traits across sites and the results showed that most traits were controlled by many genes in all the sites, indicating that selection strategies such as recurrent selection programmes can be exploited to increase the concentration of desired alleles in the base population.

Associations' studies among grain yield and its adaptive traits in hybrids involving recombinant inbred lines revealed that there were significant correlations between grain yield and its adaptive traits and among the adaptive traits. These traits can be manipulated

synchronously and conversely to improve grain yield and hybrid adaptation to different environmental conditions in western and eastern South Africa. The main direct factors contributing to yield were ear length, number of ears per plant and plant height, indicating that direct selection for ear size, prolificacy and plant height would be effective to improve grain yield of hybrids. Therefore, it can be concluded that these traits can be used as the primary selection criteria for grain yield in these respective environments and they are least negated by other traits. Grain yield was least affected by indirect factors in all the sites. In general, results show that indirect selection for most of the quantitative traits would not be effective to improve yield. However, indirect selection for early anthesis and tall plants would improve yield via silking date and improved leaf area, respectively.

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Chapter 5: Assessment of cultivar superiority of testcrosses involving recombinant maize inbred lines

ABSTRACT

Maize production and productivity is faced with several constraints such as increased levels of biotic and abiotic stresses. Therefore, there is a need for breeding and selection of superior cultivars which are stable and productive. The objectives of this study were to determine cultivar superiority of testcrosses involving recombinant maize inbred lines (RILs) and to select the best cultivar within and across four different environments. The experiment was laid out as augmented alpha lattice design. All quantitative data were subjected to GenStat 14th edition to predict the means and cultivar superiority. The combination of high grain yield and economic traits formed the main basis for selecting hybrids which qualified for advanced trials. Hybrid 10MAK10-1/N3 was the best hybrid at Ukulinga. Whereas T17/L83 and T11/L102 were the best hybrids at Cedara, respectively. T3/L48 was identified as the best hybrid at Dundee. Where PAN6611 and T1/L28 were identified as the best hybrids at Potchefstroom, respectively. At the three other sites developmental hybrids outperformed the standard check hybrids, indicating significant breeding progress. At Ukulinga the results showed that five hybrids were both high yielding and prolific; eight hybrids produced high grain yield and low grain moisture content; and nine hybrids produced lower ear aspect and higher grain yield. Whereas in Cedara seven hybrids produced high grain yield and higher number of ears per plant; six hybrids produced high grain yield and lower grain moisture; and eleven hybrids produced lower ear aspect and high grain yield. At Dundee, nine hybrids had high grain yield and number of ears per plant. While at Potchefstroom ten hybrids produced higher grain yield and number of ears per plant; five hybrids had high grain yield and lower grain moisture; and six hybrids had higher grain yield and lower ear aspect. The results showed that there is variation in the performance of high yielding hybrids within all the sites. The hybrids which fitted in the desired matrix were selected for advancement in the next programme. Hybrids 11C2340, 11C2234, 11C2252, 11C2316 and PAN6611 were the top 5 superior and stable hybrids across the sites, respectively.

Keywords: cultivar superiority, economic traits, maize, relative yield, scatter plots

5.1 INTRODUCTION

Maize plays a significant role in human and livestock nutrition worldwide (Banik et al., 2010). However, during the last two decades, production and productivity have lagged behind population growth for several reasons, including increased levels of biotic and abiotic constraints (Badu-Apraku et al., 2011). Global warming and its associated effects have changed weather patterns leading to erratic and unreliable amount and distribution of rainfall, resulting in drought (Badu-Apraku et al., 2011). Therefore, these challenges call for breeding and selection of new superior cultivars which are better able to withstand such erratic weather conditions. It pays to spend some time choosing the best hybrids (Nafziger, 2008).

Cultivar superiority can be estimated based on grain yield and related economic traits such as higher ear prolificacy, ear aspect and low grain moisture content at harvest. Harvest grain moisture can be used as a measure of maturity, and it is very rare to find trials in which later hybrids yield more (Nafziger, 2008). Therefore, the earlier the hybrid the better; hence, lower grain moisture is desirable at harvest because it is a sign of earliness. The selection for early hybrids is crucial given that climate change effects might result in shrinkage of the growing seasons in South Africa. High ear prolificacy is desired because in dry environments farmers tend to use wider spacing, and the lower number of plants can be compensated by higher ear prolificacy and hence higher grain yield. On the other hand, lower ear aspect is desirable for ease of marketability. The specific adaptability of a cultivar can be identified by plotting the maximum and the test cultivar responses on location means (Lin and Binns, 1988). All checks are required to be included at all test locations and the breeder's selections can be compared with these checks (Lin and Binns, 1988). Hence, it is essential to compare the hybrid performance with the commercial checks when determining cultivar superiority.

The general objective of the study was to determine cultivar superiority of testcrosses involving recombinant maize inbred lines and the specific objective was to select the best cultivars within and across four different environments. The identified hybrids will be

recommended for advanced trials to be planted across 8 to 10 locations across South Africa during 2013 to 2015 seasons.

5.2 MATERIALS AND METHODS

5.2.1 Germplasm

The germplasm is described in chapter 3. The commercial standard check hybrids used in the study were; CRN3505, SC633, PAN6611, PAN6Q445B, PAN6227, PAN67 and PAN53.

5.2.2 Experimental Design and Management

Trials were laid out and managed as described in chapter 2 and 3.

5.2.3 Data Collection

The following data were collected at all the four sites in accordance with protocols used by CIMMYT (Magorokosho et al., 2009):

- Grain yield ($t\ ha^{-1}$): measured by weighing the grain and ears and was adjusted to 12.5% grain moisture content.
- Number of ears per plant: measured by counting the number of ears with at least one fully developed grain and divide by the number of harvested plants
- Grain moisture content (%): measured as percentage water content of grain at harvest.
- Ear height (cm): measured as height between the base of a plant to the insertion of the uppermost ear of the same plant.
- Plant height (cm): measured as the distance between the base of a plant to the insertion point of the uppermost ear. It was measured when all the plants had flowered, since plants reach their maximum height at flowering.
- Ear position (ratio): measured as the ratio of ear height to plant height. Small values less than 0.50 indicate a low ear position and large values (>0.50) indicate high ear position.
- Ear aspect (score): cob rating on a 1-5 scale, where 1 is excellently looking and 5 is very bad looking.
- Plant number: number of plants harvested per plot.

5.2.4 Data Analysis

The data was analysed using SAS version 9.2 (SAS Institute Inc, 2010) following a general linear model (GLM) procedure:

$$Y_{ijk} = \mu + G_i + \beta_j + S_k + e_{ijk}$$

Where; Y_{ijk} = yield; μ = overall population mean; G_i = hybrids/entries; β_j = blocks; S_k = site; and e_{ijk} = random error. The entries were fixed and blocks were considered random.

The scatter plots for hybrids and environmental scores were computed from GenStat 14th Edition (Payne et al., 2011). Stability coefficients displaying cultivar superiority were also computed on GenStat 14th Edition (Payne et al., 2011).

Stability of the hybrids across the environments were estimated by cultivar superiority index (P_i) in accordance with Lin and Binns (1988) as follows:

$$P_i = \sum_{j=1}^n (X_{ij} - M_j)^2 / 2n$$

Where; n = number of locations; X_{ij} = yield of the i^{th} cultivars in the j^{th} environment; M_j = maximum yield recorded in the j^{th} environment.

The relative yield was calculated using the following formula:

$$\text{Relative Yield (\%)} = (Y/\hat{Y}_p) \times 100$$

Where; Y = adjusted grain yield; \hat{Y}_p = trial mean, mean of the checks or mean of the best check.

Adjusted yield is estimated yield obtained from the statistical software outputs after actual yield analysis.

5.3 RESULTS

5.3.1 Rainfall and temperature data during the growing season 2011/12

The rainfall data from November 2011 to April 2012 for all the sites is indicated in section 4.2. On average Potchefstroom had the highest temperature, followed by Ukulinga, Dundee and Cedara, respectively.

5.3.2 Selection of the best cultivar within four different sites

This study was aiming to identify 20 hybrids showing promising yield. Baseline yield should be top 20 among 1012 hybrids tested. High grain yield is combined with grain moisture, ear aspect and number of ears per plant, respectively; these traits are considered economic because farmers in South Africa will not accept a hybrid without these complimentary traits in their respective environments.

5.3.3 Yield vs relative yield

The results for Ukulinga are presented in Table 5-1. Hybrid 10MAK10-1/N3 had the highest adjusted and relative yield followed by T4/L73, T17/L105, 09MAK2-67/N3 and T3/L46, respectively. All the checks were ranked below the top 20; however, PAN6Q445B was highly ranked among the checks followed by PAN6227, SC633, PAN6611, PAN53 and PAN67, respectively.

The results for Cedara are displayed in Table 5-3. Hybrid T17/L83 had the highest adjusted and relative yield followed by T15/L36, T14/L101, T11/L102 and T1/L22, respectively. All the checks were ranked below the top 20; however, PAN6Q445B had the highest yield and relative yield followed by PAN6611 and PAN6227, respectively.

The results for Dundee are summarised in Table 5-5. Hybrids T13/L16 and T3/L48 had the highest adjusted and relative yield followed by T17/L112, T1/L65 and T3/L47, respectively. All the checks were ranked below the top 20; however, PAN6Q445B was the highly ranked followed by PAN6611 and PAN6227.

The results for Potchefstroom are shown in Table 5-7. PAN6611 had the highest adjusted and relative yield followed by T1/L28, 10MAK10-27/10MAK9-34, CRN3505 and T15/L15, respectively. Among the checks, only SC633 was ranked below the top 20.

5.3.4 Yield vs economic traits

The results for Ukulinga are shown in Table 5-2. Hybrid 10MAK10-1/N3 had the highest number of ears per plant among the top 20 hybrids; whereas its grain moisture and ear aspect score were the third lowest among the top 20 hybrids. Among the checks,

PAN6Q445B had the highest number of ears per plant; whereas its grain moisture was the third lowest after SC633 and PAN53; and its ear aspect was the lowest among the checks.

The results for Cedara are depicted in Table 5-4. Hybrid T17/L83 had the lowest number of ears per plant, fourth highest grain moisture content and average ear aspect score. Among the top 5 hybrids, T11/L102 which was ranked fourth had the highest number of ears per plant, lowest grain moisture and third lowest ear aspect score. Among the checks, PAN6Q445B had the highest number of ears per plant, highest grain moisture followed by PAN6611 and PAN6227, and it also had the lowest ear aspect score.

The results for Dundee are presented in Table 5-6. Hybrid T3/L48 had the highest number of ears per plant; whereas hybrid T13/L16 had the seventh highest number of ears per plant. Among the checks, PAN6Q445B had the highest number of ears per plant followed by PAN6227 and PAN6611, respectively.

The results for Potchefstroom are displayed in Table 5-8. Among the top 5 hybrids, T1/L28 had the highest number of ears per plant, lowest grain moisture and lowest ear aspect score. Among the checks, PAN6611 and CRN3505 had the highest number of ears per plant followed by SC633; however SC633 displayed the lowest grain moisture content followed by CRN3505 and PAN6611; but in terms of ear aspect, PAN6611 had the lowest followed by SC633 and CRN3505.

Table 5-1: Hybrid rank at Ukulinga research farm with respect to adjusted, actual and relative yield

Entry	Name	Yield (t ha ⁻¹)		Yield (rank)		Relative Yield (%)		
		Adj.	Actual	Adj.	Actual	Mean Check	Best Check	Trial Mean
917	10MAK10-1/N3	10.0	9.1	1	1	199	147	250
610	11C1966	8.9	8.1	2	3	179	132	224
902	11C2260	8.8	6.7	3	28	177	131	222
756	09MAK2-67/N3	8.7	6.5	4	36	174	129	218
391	11C1745	8.6	8.1	5	4	173	128	217
209	11C1563	8.5	8.0	6	5	171	126	214
269	11C1623	8.4	6.2	7	55	169	125	211
547	11C1903	8.3	6.1	8	60	167	123	209
361	11C1715	8.3	6.1	9	65	165	122	207
299	11C1653	8.2	6.0	10	70	163	121	204
838	11C2196	8.2	6.0	11	71	163	121	204
483	11C1838	8.0	7.4	12	6	160	118	200
948	11C2306	8.0	5.8	13	91	160	118	200
291	11C1645	7.9	5.7	14	100	158	117	198
555	09MAK2-123/P1	7.9	5.7	15	102	158	117	197
134	11C1486	7.9	5.7	16	103	158	117	197
102	11C1454	7.7	5.5	17	130	154	114	193
309	11C1663	7.5	6.7	18	24	151	111	189
390	09MAK2-79/P1	7.4	5.2	19	176	149	110	186
614	11C1970	7.4	5.2	20	186	147	109	185
Control hybrids								
1020	PAN6Q445B	6.8	6.7	52	22	135	100	169
1010	PAN6227	6.3	4.2	82	434	127	94	159
966	SC633	4.6	4.5	326	343	92	68	116
1021	PAN6611	4.5	4.8	355	269	90	67	113
155	PAN53	4.3	4.3	409	382	86	64	108
193	PAN67	3.7	4.9	579	237	73	54	92
Summary statistics								
	Mean (Checks)	5.03						
	P-value	0.715						
	SED	2.562						
	CV (%)	33.57						
	Mean	3.99						
	Max	9.96						
	Min	-0.209						

Adj. = adjusted yield and rank, respectively. SED = standard error difference. CV = coefficient of variation. Max = maximum. Min = minimum.

Table 5-2: Hybrid rank at Ukulinga research farm with respect to adjusted yield and economic traits

Entry	Name	Economic Traits		
		EPP	GM	EA
917	10MAK10-1/N3	1.7	13.6	2.0
610	11C1966	1.4	14.8	2.0
902	11C2260	1.2	14.4	3.6
756	09MAK2-67/N3	1.3	13.4	2.6
391	11C1745	1.1	14.3	0.8
209	11C1563	1.1	13.7	0.8
269	11C1623	1.3	14.4	2.6
547	11C1903	1.2	12.6	4.1
361	11C1715	1.1	13.7	2.6
299	11C1653	1.1	13.7	3.1
838	11C2196	1.2	13.1	2.1
483	11C1838	1.1	15.8	0.8
948	11C2306	1.2	14.1	2.1
291	11C1645	1.2	14.5	2.1
555	09MAK2-123/P1	1.2	13.4	2.1
134	11C1486	1.1	15.0	1.6
102	11C1454	1.1	14.5	3.1
309	11C1663	1.2	15.4	2.0
390	09MAK2-79/P1	1.7	14.8	2.6
614	11C1970	1.1	14.4	2.1
Control hybrids				
1020	PAN6Q445B	1.3	14.0	2.5
1010	PAN6227	1.1	15.3	3.1
966	SC633	1.1	12.5	2.8
1021	PAN6611	1.0	14.1	2.7
155	PAN53	1.0	13.7	3.7
193	PAN67	0.9	14.5	2.7
Summary statistics				
	P-value	0.19	0.15	0.486
	SED	0.233	0.967	1.199
	CV (%)	16.75	5.64	24.84
	Mean	1.04	13.44	3.00
	Max	2.0	16.8	5.7
	Min	0.4	10.1	0.8

EPP = number of ears per plant. GM = grain moisture. EA = ear aspect. SED = standard error difference. CV = coefficient of variation. Max = maximum. Min = minimum.

Table 5-3: Hybrid rank at Cedara with respect to adjusted, actual and relative yield

Entry	Name	Yield (t/ha)		Yield (rank)		Relative yield (%)		
		Adj.	Actual	Adj.	Actual	Mean Check	Best Check	Trial Mean
698	11C2055	15.5	13.0	1	7	157	128	192
305	11C1659	15.1	12.6	2	11	153	125	188
864	11C2222	15.0	12.8	3	8	152	125	186
876	11C2234	14.9	12.7	4	10	151	124	185
180	11C1534	14.4	15.3	5	1	146	119	179
977	11C2336	14.3	11.8	6	22	145	119	178
782	11C2139	14.3	11.8	7	25	145	118	177
279	11C1633	14.1	11.9	8	20	143	117	175
196	11C1550	14.1	13.6	9	4	143	117	175
786	11C2143	14.0	13.5	10	5	142	116	174
420	11C1774	14.0	13.5	11	6	141	116	173
270	11C1624	13.7	11.2	12	50	139	114	170
223	11C1577	13.5	14.3	13	2	136	112	167
187	11C1541	13.4	11.2	14	48	136	111	166
216	11C1570	13.3	10.8	15	64	135	111	165
486	11C1841	13.3	10.8	16	65	135	111	165
699	11C2056	13.3	11.1	17	53	135	110	165
585	11C1941	13.3	12.6	18	12	134	110	165
335	11C1689	13.1	10.6	19	87	132	108	162
494	11C1849	13.0	10.5	20	94	131	108	161
Control hybrids								
1011	PAN6Q445B	12.1	12.1	52	18	122	100	150
1012	PAN6611	9.7	9.7	255	186	98	81	120
1010	PAN6227	7.9	7.1	544	714	80	65	98
Summary statistics								
	Mean (Checks)	9.9						
	P-value	0.042						
	SED	1.982						
	CV (%)	24.3						
	Mean	8.06						
	Max	15.5						
	Min	0.2						

Adj. = adjusted yield and rank, respectively. SED = standard error difference. CV = coefficient of variation. Max = maximum. Min = minimum.

Table 5-4: Hybrid rank at Cedara with respect to economic traits

Entry	Name	Economic traits		
		EPP	GM	EA
698	11C2055	1.0	16.5	2.4
305	11C1659	1.6	17.3	1.9
864	11C2222	1.7	16.0	1.1
876	11C2234	2.7	15.1	1.6
180	11C1534	1.8	16.4	1.4
977	11C2336	2.3	15.0	3.9
782	11C2139	1.9	16.9	1.9
279	11C1633	1.5	14.6	1.6
196	11C1550	1.5	17.4	2.1
786	11C2143	1.7	16.3	3.1
420	11C1774	2.0	16.8	1.6
270	11C1624	2.0	14.3	2.9
223	11C1577	2.0	15.4	1.6
187	11C1541	1.5	16.2	2.6
216	11C1570	1.4	17.2	2.9
486	11C1841	1.8	17.3	3.4
699	11C2056	3.2	13.5	2.1
585	11C1941	1.0	14.8	3.1
335	11C1689	1.9	15.8	2.4
494	11C1849	1.6	16.5	2.4
Control hybrids				
1011	PAN6Q445B	1.9	16.1	1.9
1012	PAN6611	1.5	15.3	2.8
1010	PAN6227	1.3	14.1	3.4
Summary statistics				
	Mean (Checks)	1.56		
	P-value	0.516	0.247	0.154
	SED	0.614	1.618	0.930
	CV (%)	27.2	7.6	25.0
	Mean	1.36	15.43	2.99
	Max	4.4	18.2	5.6
	Min	0.0	11.4	0.6

EPP = number of ears per plant. GM = grain moisture. EA = ear aspect. SED = standard error difference. CV = coefficient of variation. Max = maximum. Min = minimum.

Table 5-5: Hybrid rank at Dundee with respect to adjusted, actual mean and relative yield

Entry	Name	Yield (t/ha)		Yield (rank)		Relative Yield (%)		
		Adj.	Actual	Adj.	Actual	Mean Check	Best Check	Trial Mean
144	11C1497	11.8	8.9	1	11	227	170	295
410	11C1764	11.8	9.1	2	7	227	170	295
955	11C2313	11.8	8.9	3	15	227	170	295
542	11C1898	10.7	7.8	4	33	206	154	268
400	11C1754	10.3	7.3	5	48	198	148	257
472	11C1827	10.3	7.6	6	39	198	148	257
501	11C1856	10.3	7.3	7	49	198	148	257
892	11C2250	10.1	7.1	8	66	194	145	252
27	11C1377	9.9	6.9	9	70	190	142	246
124	11C1476	9.9	6.9	10	72	190	142	246
437	11C1792	9.7	6.9	11	84	186	139	241
912	11C2270	9.7	6.7	12	107	186	139	241
698	11C2055	9.4	8.6	13	18	181	136	235
934	11C2292	9.4	6.7	14	110	181	136	235
949	11C2307	9.4	6.7	15	111	181	136	235
286	11C1640	9.2	6.3	16	141	177	133	230
457	11C1812	9.2	9.1	17	8	177	133	230
688	11C2045	9.2	6.3	18	151	177	133	230
844	11C2202	9.0	8.2	19	27	173	130	225
187	11C1541	8.6	5.6	20	211	165	123	214
Control hybrids								
1011	PAN6Q445B	7.0	7.0	85	69	134	100	173
1012	PAN6611	5.7	5.7	191	205	109	81	141
1010	PAN6227	3.0	6.0	730	178	57	43	74
Summary statistics								
Mean (Checks)	5.2							
P-values	0.795							
SED	4.004							
CV (%)	50.2							
Mean	4.01							
Max	11.8							
Min	-2.2							

Adj. = adjusted yield and rank, respectively. SED = standard error difference. CV = coefficient of variation. Max = maximum. Min = minimum.

Table 5-6: Hybrid rank at Dundee with respect to adjusted yield, actual yield and economic traits

Entry	Name	Yield (t/ha)		Yield (rank)		Economic Trait
		Adj.	Actual	Adj.	Actual	EPP
144	11C1497	11.8	8.9	1	11	1.8
410	11C1764	11.8	9.1	2	7	2.6
955	11C2313	11.8	8.9	3	15	1.9
542	11C1898	10.7	7.8	4	33	2.4
400	11C1754	10.3	7.3	5	48	2.1
472	11C1827	10.3	7.6	6	39	2.6
501	11C1856	10.3	7.3	7	49	1.8
892	11C2250	10.1	7.1	8	66	1.9
27	11C1377	9.9	6.9	9	70	2.2
124	11C1476	9.9	6.9	10	72	1.9
437	11C1792	9.7	6.9	11	84	2.0
912	11C2270	9.7	6.7	12	107	1.6
698	11C2055	9.4	8.6	13	18	1.5
934	11C2292	9.4	6.7	14	110	2.2
949	11C2307	9.4	6.7	15	111	2.0
286	11C1640	9.2	6.3	16	141	1.8
457	11C1812	9.2	9.1	17	8	1.6
688	11C2045	9.2	6.3	18	151	2.0
844	11C2202	9.0	8.2	19	27	1.5
187	11C1541	8.6	5.6	20	211	1.6
Control hybrids						
1011	PAN6Q445B	7.0	7.0	85	69	2.2
1012	PAN6611	5.7	5.7	191	205	1.3
1010	PAN6227	3.0	6.0	730	178	1.4
Summary statistics						
Mean (Checks)		5.2				
P-values		0.795				0.168
SED		4.004				0.617
CV (%)		50.2				36.1
Mean		4.01				1.35
Max		11.8				3.9
Min		-2.2				-0.4

Adj. = adjusted yield and rank, respectively. EPP = number of ears per plant. SED = standard error difference. CV = coefficient of variation. Max = maximum. Min = minimum.

Table 5-7: Hybrid rank at Potchefstroom with respect to adjusted, actual and relative yield

Entry	Name	Yield (t/ha)		Yield (rank)		Relative yield (%)		
		Adj.	Actual	Adj.	Actual	Mean Check	Best Check	Trial Mean
132	PAN6611	8.8	8.8	1	1	112	100	134
110	11C1579	8.7	8.2	2	4	111	99	132
11	10HDTX11	8.5	8.0	3	9	109	97	130
131	CRN3505	8.4	8.4	4	2	107	96	128
124	11C1483	8.4	8.0	5	8	107	95	128
38	10HDTX32	8.3	8.1	6	5	106	95	127
103	11C2245	8.2	7.8	7	12	104	93	125
62	11C2243	8.1	7.8	8	11	104	93	124
7	10HDTX45	8.1	7.7	9	14	104	92	124
101	11C1350	7.9	7.4	10	26	100	89	120
1	10HDTX52	7.8	7.4	11	23	100	89	119
84	11C2242	7.7	8.3	12	3	99	88	118
25	10HDTX7	7.7	7.8	13	10	98	88	117
108	11C1511	7.6	7.6	14	18	97	87	116
102	11C2226	7.6	7.7	15	13	97	86	116
128	11C1554	7.6	8.1	16	6	96	86	115
52	11C2258	7.5	8.1	17	7	96	86	115
79	11C1738	7.5	7.0	18	40	96	86	115
100	11C1832	7.5	7.2	19	32	96	86	115
33	10HDTX47	7.5	7.4	20	21	96	85	114
Control hybrids								
132	PAN6611	8.8	8.8	1	1	112	100	134
131	CRN3505	8.4	8.4	4	2	107	96	128
4	SC633	6.3	6.2	72	83	81	72	96
Summary statistics								
	MEAN (Checks)	7.8						
	P-value	<0.001						
	CV (%)	16.9						
	SED	0.971						
	Mean	6.56						
	Max	8.8						
	Min	4.2						

Adj. = adjusted yield and rank, respectively. SED = standard error difference. CV = coefficient of variation. Max = maximum. Min = minimum

Table 5-8: Hybrid rank at Potchefstroom with respect to adjusted yield and economic traits

Entry	Name	Economic traits		
		EPP	GM	EA
132	PAN6611	2.3	13.0	1.9
110	11C1579	2.6	12.4	1.8
11	10HDTX11	2.6	13.8	2.8
131	CRN3505	2.3	12.7	2.8
124	11C1483	2.8	13.3	1.5
38	10HDTX32	2.6	14.4	2.8
103	11C2245	2.6	13.1	2.8
62	11C2243	2.2	12.0	2.5
7	10HDTX45	2.2	13.2	2.8
101	11C1350	2.3	14.2	1.8
1	10HDTX52	2.6	13.9	2.8
84	11C2242	2.4	12.8	2.3
25	10HDTX7	2.0	13.0	2.8
108	11C1511	2.4	12.3	2.5
102	11C2226	2.1	13.0	1.8
128	11C1554	2.1	12.2	3.3
52	11C2258	1.7	13.6	3.3
79	11C1738	2.1	13.4	3.8
100	11C1832	2.5	12.1	2.5
33	10HDTX47	2.4	12.3	2.5
Control hybrids				
132	PAN6611	2.3	13.0	1.9
131	CRN3505	2.3	12.7	2.8
4	SC633	1.6	11.7	2.7
Summary statistics				
MEAN (Checks)		7.8		
P-value		0.008	0.003	<0.001
CV (%)		14.5	6.8	30.1
SED		0.335	0.871	0.738
Mean		2.10	12.76	3.13
Max		2.8	15.0	5.3
Min		1.4	10.6	1.5

EPP = number of ears per plant. GM = grain moisture. EA = ear aspect. SED = standard error of difference. CV = coefficient of variation. Max = maximum. Min = minimum.

5.3.5 Distribution of the top 25 high yielding hybrids in relation to grain yield and selected yield adaptive traits

UKULINGA

Yield vs number of ears per plant

The results are exhibited in Figure 5-1. The hybrids which were fitted in quadrant B are desired and will be considered for advancement. The results show that 5 hybrids were both high yielding and prolific. Hybrid 1 (10MAK10-1/N3) produced the highest grain yield and number of ears per plant. Hybrid 2 (11C1966) produced the second highest grain yield and number of ears per plant. Five hybrids (27, 28, 29, 30 and 31) produced the lowest grain yield and number of ears per plant.

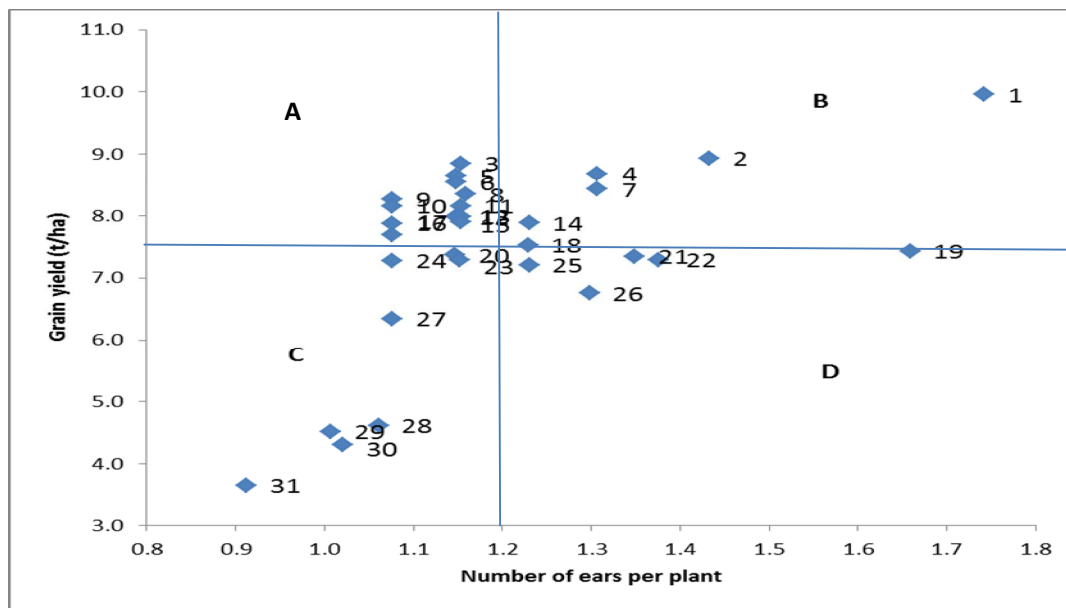


Figure 5-1: Hybrid distribution with respect to number of ears per plant and grain yield

Yield vs grain moisture

The results are presented in Figure 5-2. Hybrids which were fitted in quadrant A are desired and will be considered for advancement. Eight hybrids produced high grain yield and low grain moisture. Among these hybrids; hybrid 1 (10MAK10-1/N3) had the highest grain yield; whereas hybrid 8 (T17/L65) had the lowest grain moisture. Three hybrids produced lower

grain moisture and lower grain yield; whereas two hybrids (27 and 31) produced higher grain moisture and lower grain yield.

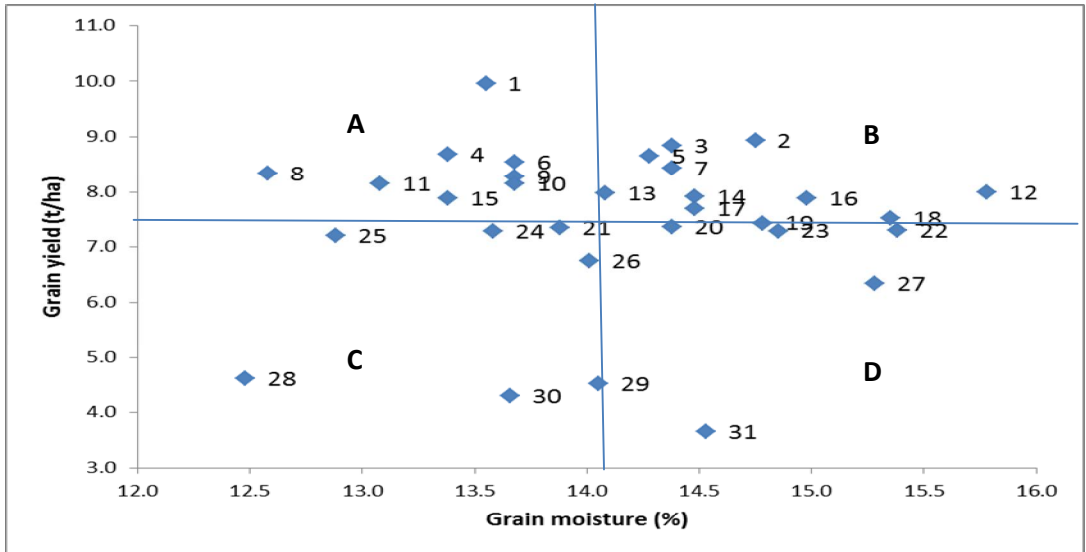


Figure 5-2: Hybrid distribution with respect to grain moisture and grain yield

Yield vs ear aspect

The results are shown in Figure 5-3. Hybrids which were fitted in quadrant A are desired and will be considered for advancement. Ten hybrids produced lower ear aspect and higher grain yield. Among these hybrids, hybrid 1 (10MAK10-1/N3) produced the highest grain yield; whereas hybrid 5, 6 and 12 produced the lowest ear aspect. Seven hybrids produced the lowest grain yield and highest ear aspect score.

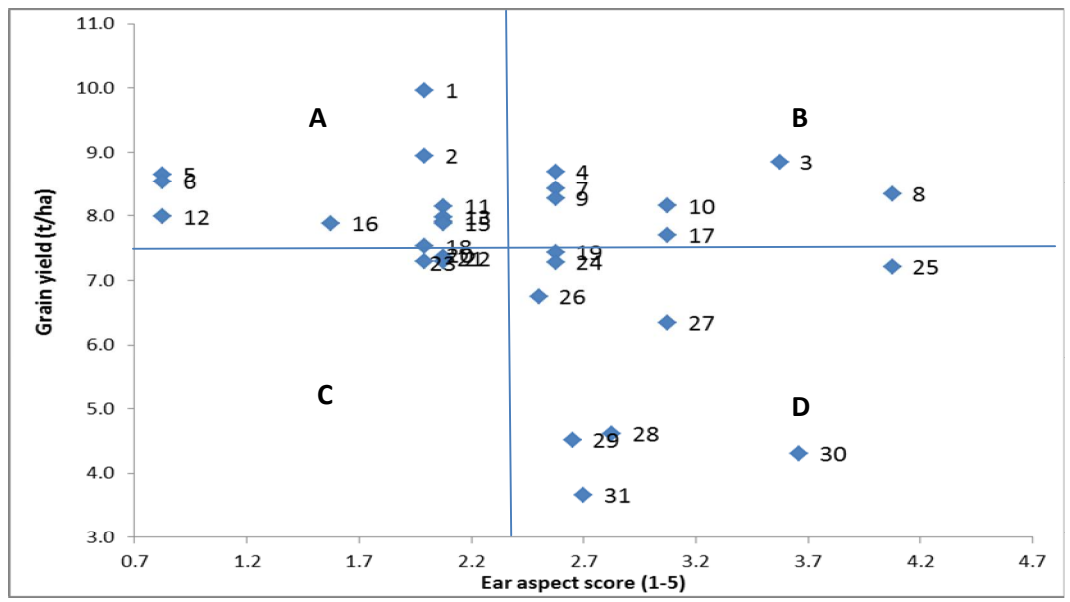


Figure 5-3: Hybrid distribution with respect to ear aspect and grain yield

CEDARA

Yield vs number of ears per plant

The results are displayed in Figure 5-4. Hybrids which were fitted in quadrant B are desired and will be considered for advancement. Seven hybrids produced high grain yield and higher number of ears per plant. Among those hybrids; hybrid 17 (T14/L83) had the highest number of ears per plant, while hybrids 4 (T11/L102) and 6 (T15/L115) had the highest grain yield, respectively. Four hybrids produced both the lowest grain yield and number of ears per plant.

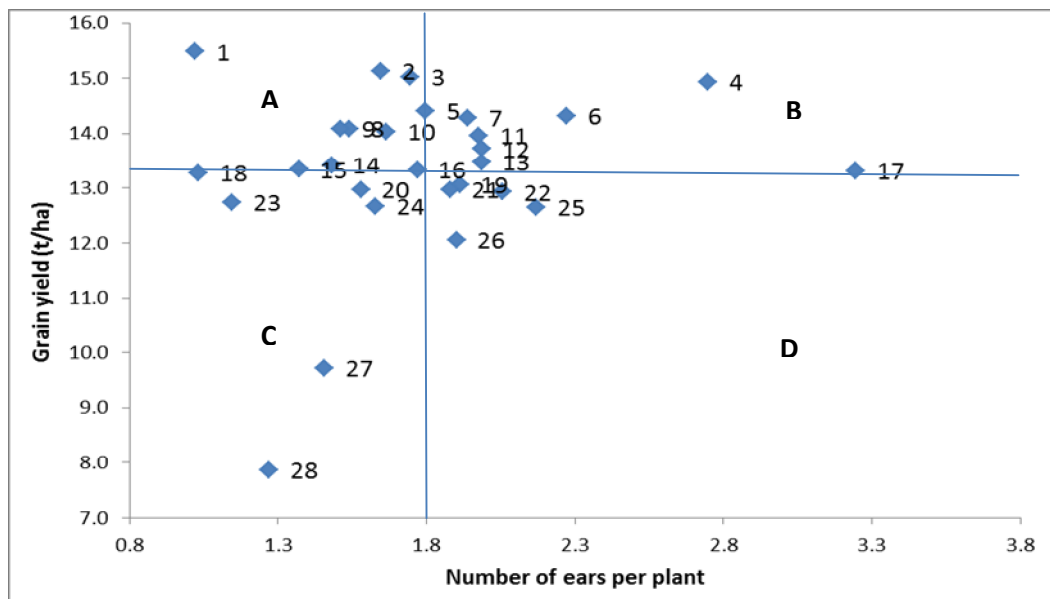


Figure 5-4: Hybrid distribution with respect to number of ears per plant and grain yield

The results are depicted in Figure 5-5. Hybrids which were fitted in quadrant A are desired and will be selected for advancement. Seven hybrids produced high grain yield and lower grain moisture. Among these hybrids, hybrid 4 (T11/L102) had the highest grain yield; whereas hybrid 17 (T14/L83) had the lowest grain moisture. Three hybrids had the lowest grain moisture and grain yield.

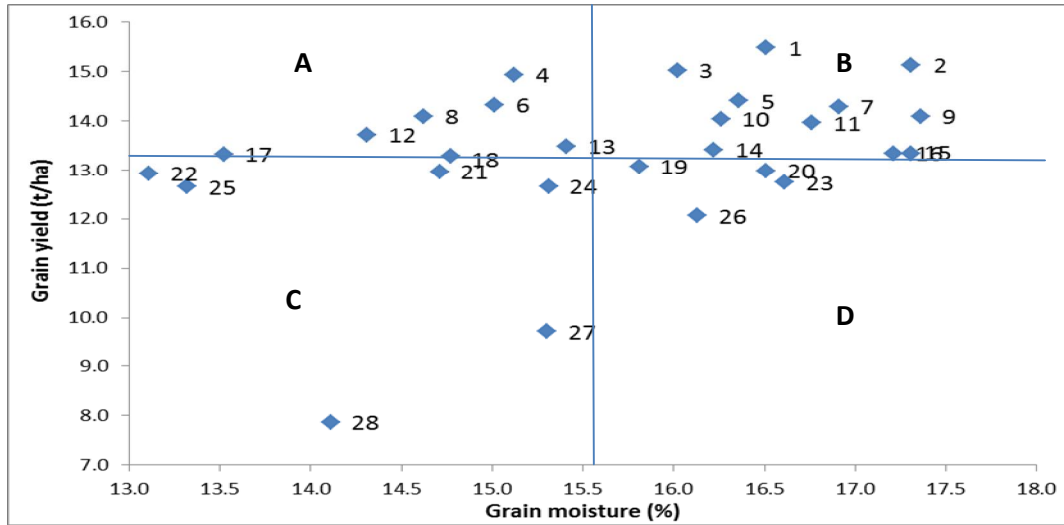


Figure 5-5: Hybrid distribution with respect to grain moisture and grain yield

Yield vs ear aspect

The results are exhibited in Figure 5-6. Hybrids which were fitted in quadrant A are desired and will be considered for advancement. Eleven hybrids showed lower ear aspect score and high grain yield. Among these hybrids, hybrid 1 (T17/L83) had the highest grain yield; whereas hybrids 3 (T14/L101) and 5 (T1/L22) had the lowest ear aspect, respectively. Two hybrids had the lowest grain yield and high ear aspect.

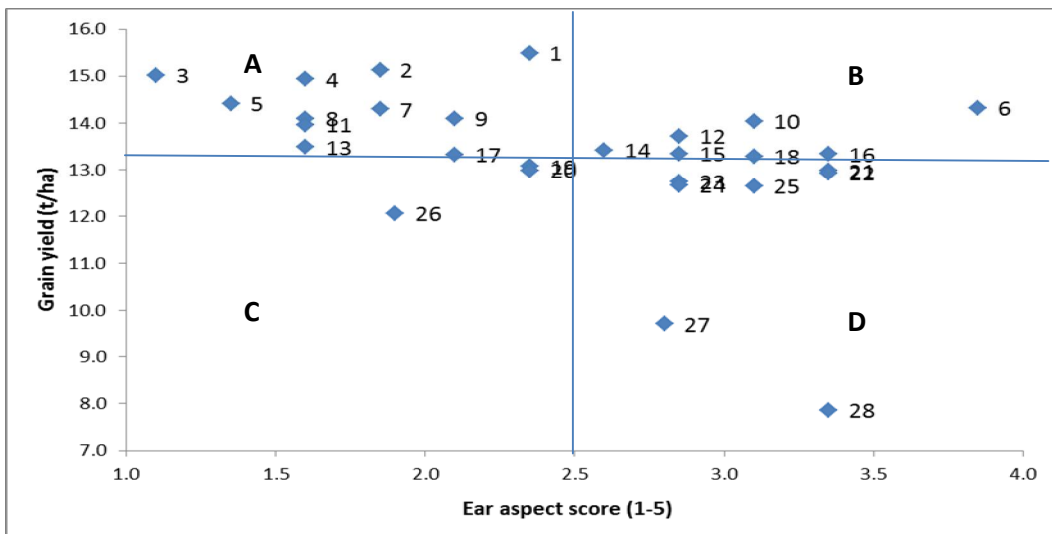


Figure 5-6: Hybrid distribution with respect to ear aspect and grain yield

DUNDEE

Yield vs number of ears per plant

The results are presented in Figure 5-7. Hybrids which were fitted in quadrant B are desired and will be considered for advancement. Ten hybrids had high grain yield and number of ears per plant. Among these hybrids, hybrid 2 (T3/L48) had the highest grain yield and number of ears per plant followed by hybrid 4 (T1/L65) with high grain yield but its number of ears per plant was not higher than that of hybrid 6 (T4/L56) which also had higher grain yield. Five hybrids had the lowest number of ears per plant and grain yield.

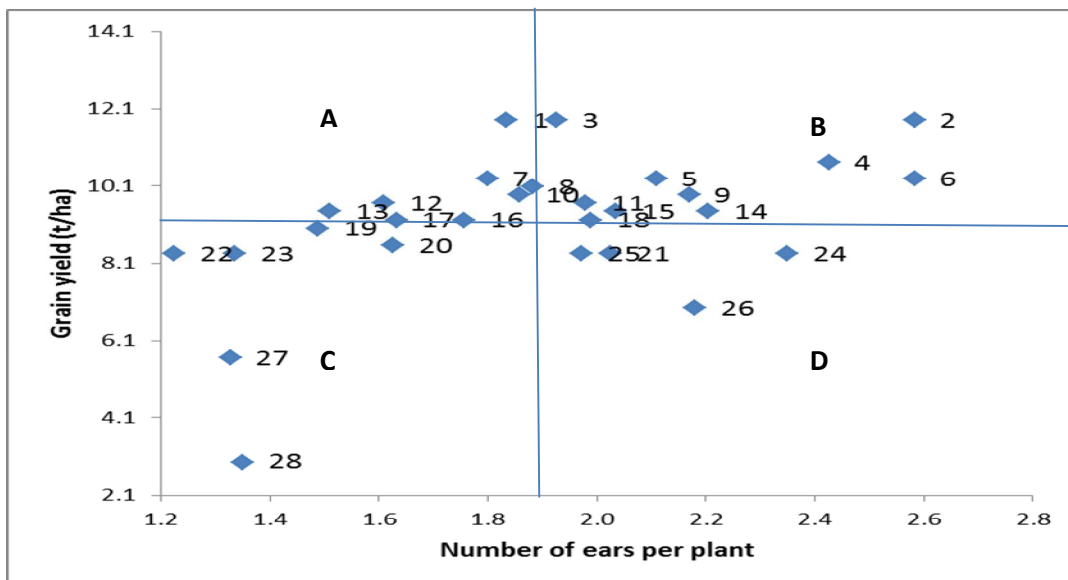


Figure 5-7: Hybrid distribution with respect to number of ears per plant and grain yield

POTCHEFSTROOM

Yield vs number of ears per plant

The results are shown in Figure 5-8. Hybrids which were fitted in quadrant B are desired and will be selected for advancement. Ten hybrids produced higher grain yield and number of ears per plant. Among these hybrids, hybrid 1 (PAN6611), 2 (T1/L28), 3 (10MAK10-27/10MAK9-34), 4 (CRN3505), 5 (T15/L15), 6 (N3/10MAK9-32) and 7 (T1/L104) had the highest grain yield, respectively; whereas hybrid 5 (T15/L15) had the highest number of ears per plant. Hybrid 26 (SC633) had the lowest grain yield and number of ears per plant.

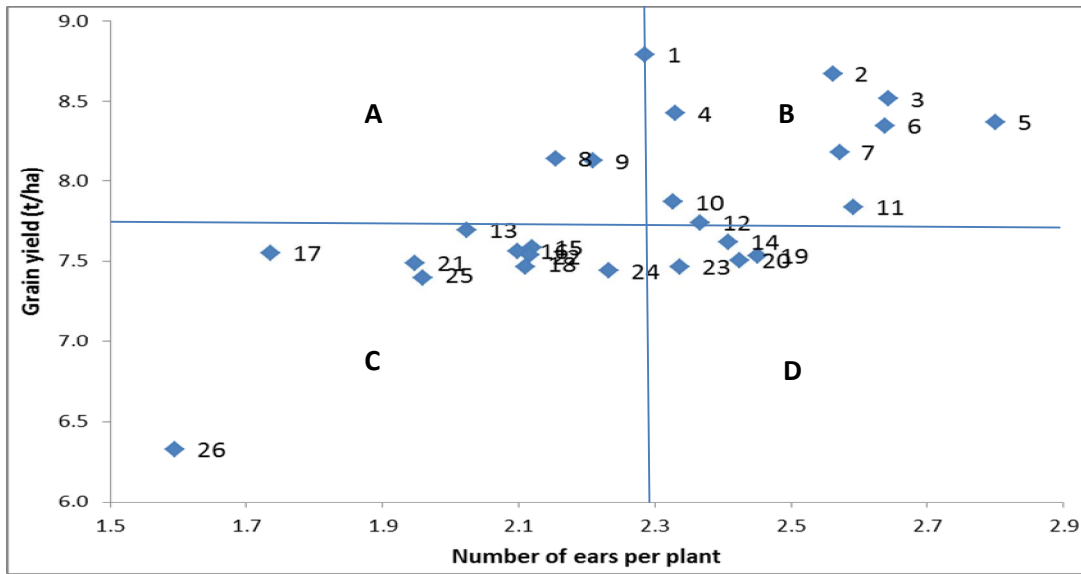


Figure 5-8: Hybrid distribution with respect to number of ears per plant and grain yield

Yield vs grain moisture

The results are displayed in Figure 5-9. Hybrids which were fitted in quadrant A are desired and will be considered for advancement. Five hybrids had high grain yield and lower grain moisture. Among these hybrids, hybrids 1 (PAN6611), 2 (T1/L28) and 4 (CRN3505) had the highest grain yield; whereas hybrid 8 (T11/L103) had the lowest grain moisture. Hybrid 26 (SC633) had the lowest grain yield and grain moisture.

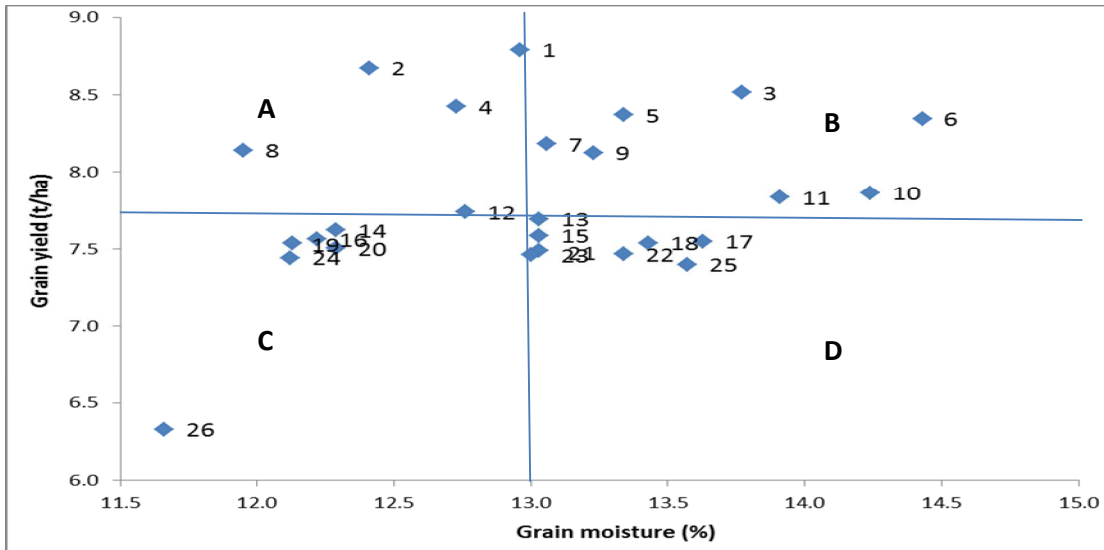


Figure 5-9: Hybrid distribution with respect to grain moisture and grain yield

Yield vs ear aspect

The results are depicted in Figure 5-10. Hybrids which were fitted in quadrant A are desired and will be selected for advancement. Six hybrids had higher grain yield and lower ear aspect score. Among these hybrids, hybrids 1 (PAN6611), 2 (T1/L28) and 5 (T15/L15) had the highest grain yield; whereas hybrid 5 (T15/L15) had the lowest ear aspect. Seven hybrids had the lowest grain yield and highest ear aspect.

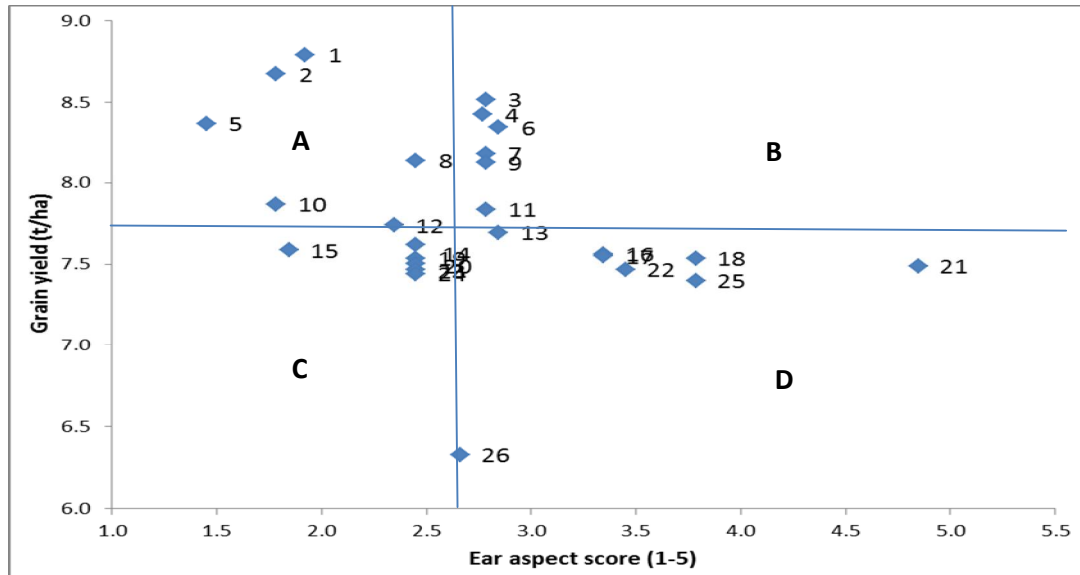


Figure 5-10: Hybrid distribution with respect to ear aspect and grain yield

5.3.6 Selection of the best cultivar across four different sites

The results are exhibited in Table 5-9. Only 88 hybrids which were planted across the four sites were analysed for cultivar superiority and stability. Out of these, top 10 and bottom 10 hybrids were tabled for analysis. Cultivar superiority index ranged between 2.51 and 19.09. Hybrids 11C2340, 11C2234, 11C2252, 11C2316 and PAN6611 had the lowest superiority values, respectively and were placed at the top of the stability table. Whereas hybrids 11C2258, 11C1444, 11C2257, 11C2238 and 11C2360 had the highest superiority values and were placed at the bottom five of the stability table, respectively.

Table 5-9: Yield superiority of 20 hybrids averaged four different sites

Hybrid Name	Mean (t/ha)	Cultivar Superiority Index
Top 10		
11C2340	8.28	2.51
11C2234	7.74	3.23
11C2252	7.12	4.17
11C2316	7.03	4.50
PAN6611	7.40	4.56
11C2242	6.98	5.02
11C2335	6.54	5.65
11C1357	6.57	5.76
11C1738	6.79	5.79
11C1512	6.63	5.90
Bottom 10		
11C1385	4.44	15.95
11C1747	4.84	16.06
11C1470	4.67	16.21
11C1741	4.55	16.93
11C2301	4.40	17.37
11C2258	4.51	17.86
11C1444	4.18	17.92
11C2257	4.16	18.21
11C2238	3.93	18.98
11C2360	4.24	19.09

5.4 DISCUSSION

5.4.1 Selection of the best cultivar within four different sites

In this study, high number of ears per plant, low grain moisture and lower ear aspect are desired since they depict ear prolificacy, earliness to physiological maturity and high quality ears, respectively which are the fundamental characteristics of a desired commercial hybrid in South Africa.

UKULINGA

Hybrid 10MAK10-1/N3 had the highest adjusted and relative yield followed by T4/L73, T17/L105, 09MAK2-67/N3 and T3/L46, respectively. The results show that 10MAK10-1/N3 was the best hybrid in terms of grain yield and relative yield. Therefore, this hybrid can be used in a breeding programme where the main objectives are higher grain yield and

prolificacy. The results show that all the checks performed poorer than the top developmental hybrids. Thus, 10MAK10-1/N3 also out yielded all the commercial hybrid checks used in the study and it was the best hybrid in terms of grain yield and relative yield.

The results show that the high grain yield exhibited by 10MAK10-1/N3 can be attributed to prolificacy. This hybrid also had other desired characteristics of a good commercial hybrid such as low grain moisture and lower ear aspect score. The results show that 10MAK10-1/N3 outperformed all the commercial hybrid checks, since it performed even beyond the best check in terms of economic traits, qualifying it as a candidate for further testing in multilocation trials throughout the country.

Hybrid 10MAK10-1/N3 was selected as the best hybrid at Ukulinga in terms of grain yield, relative yield and economic traits. Several other authors have performed cultivar superiority studies using different cultivars under different environmental conditions for different studies. Adeniyi (2011) reported that in terms of selected secondary traits TZSR-W was significantly superior to variety TZESR-W; whereas in terms of grain yield, TZESR-W was superior to TZSR-W under late season planting; hence, these results are in contrast with the current study since they show that superiority of a cultivar in secondary traits does not guarantee grain yield superiority. However, the results for the current study are in accordance with Bello et al. (2012) who reported that there was variation in performances of the outstanding hybrids, and among the superior hybrids there are those which displayed superior performance for grain yield and related secondary traits.

CEDARA

Hybrid T17/L83 had the highest adjusted and relative yield followed by T15/L36, T14/L101, T11/L102 and T1/L22, respectively. The results show that these hybrids were the best five developmental hybrids at Cedara in order of their appearance. The results show that the best five developmental hybrids performed better than all the commercial checks even the best commercial check in terms of grain yield and relative yield.

The results show that even though developmental hybrid T17/L83 was the best in terms of grain yield and relative yield, it neither was prolific, early nor exhibited the good ear aspect

indicating that it lacked the complimentary traits which farmers look for in a good hybrid in South Africa. Among the top 5 hybrids, T11/L102 which was ranked fourth in terms of grain yield had the highest number of ears per plant, lowest grain moisture and third lowest ear aspect score. Thus, T11/L102 was the most balanced developmental hybrid since it displayed all the desired characteristics of a good commercial hybrid such as outstanding prolificacy, earliness and good ear aspect, respectively. These results show that the best developmental hybrids performed better than the commercial hybrids at Cedara.

The results show that developmental hybrid T17/L83 was the best hybrid at Cedara in terms of grain yield and relative yield; however, it displayed poor performance in terms of economic traits. Whereas; T11/L102 was the most balanced hybrid with respect to grain yield, relative yield and economic traits, even though its grain yield was not higher than that of T17/L83. Therefore, the latter will be advanced for the target environments which are represented by Cedara. Several authors have also reported on cultivar superiority using different hybrids under different environmental conditions. Azeez et al. (2005) reported that hybrid 9134-14 had the superior performances in terms of grain yield and earliness under weed pressures and drought stress. These results are in contrast with the current study since they show the superiority of one cultivar for grain yield and secondary traits. However, they are in accordance with Adeniyi (2011) who reported that in terms of selected secondary traits cultivar TZSR-W was significantly superior to variety TZESR-W; whereas in terms of grain yield, TZESR-W was superior to TZSR-W under late season planting.

DUNDEE

Hybrids T13/L16 and T3/L48 had the highest adjusted and relative yield followed by T17/L112, T1/L65 and T3/L47, respectively. The results show that these were the best hybrids for grain yield and relative yield at Dundee. The results also show that the developmental hybrids performed beyond the commercial hybrid checks used in the study; hence, T13/L16, T3/L48 and T17/L112 were mutually the best hybrids in terms of grain yield and relative yield.

Hybrid T3/L48 had the highest number of ears per plant; whereas hybrid T13/L16 had the seventh highest number of ears per plant. The results have shown that T3/L48 was among

the best hybrids in terms of grain yield and relative yield, and now it is the most prolific since it had the highest number of ears per plant. This developmental hybrid possesses the characteristics of a desired commercial hybrid. Therefore, the developmental hybrids performed better than the commercial hybrid checks used in the study even the best commercial hybrid check.

The results show that T3/L48 was the best hybrid at Dundee with respect to grain yield, relative yield and prolificacy. However, there are still some traits which still need to be improved in this hybrid such as earliness and ear aspect to fulfil its selection as the best developmental hybrid at Dundee in terms of grain yield, relative yield and economic traits. Unfortunately these complimentary traits were not measured at Dundee due to some logistical reasons. Nonetheless, this hybrid will be advanced to multilocational trials across South Africa. There are different authors who have reported on a similar subject using different hybrids under different environmental conditions. These results are in accord with Gondim et al. (2006) who identified three superior cultivars out of fourteen under different abiotic stress conditions. These results are also in accordance with Magorokosho et al. (2003) who reported that there was no significant differences in grain yield performance of ZM601 and ZM607 lines; however, they reported that ZM601 was more prolific than ZM607 lines.

POTCHEFSTROOM

The results show that the best developmental hybrids were only out yielded by PAN6611 in terms of grain yield and relative yield since they managed to out yield the other two commercial hybrid checks.

The results reveal that among the top 5 best hybrids with commercial hybrid checks comprised, it is developmental hybrid T1/L28 which had all the best characteristics of a desired commercial hybrid since it displayed best results for prolificacy, earliness and ear aspect. Nevertheless, among the checks PAN6611 was the best in terms of prolificacy and earliness. Therefore, T1/L28 performed better than the best commercial hybrid check in terms of economic traits, qualifying it for advanced testing.

The results show that PAN6611 was the best hybrid in terms of grain yield and relative yield followed by developmental hybrid T1/L28 at Potchefstroom. However, developmental hybrid T1/L28 was the best hybrid in terms of earliness, prolificacy and ear aspect. Several authors have also reported on similar studies using different hybrids under different environments. Has et al. (2012) studied 264 maize accessions for early maturity and high grain dry matter, and they reported that three hybrids were superior for grain yield and were early; hence, these results are in accordance with the current study since the best developmental hybrid was among the two superior hybrids for grain yield and it was early.

5.4.2 Selection of the best cultivar across four different sites

The results show that hybrids 11C2340, 11C2234, 11C2252, 11C2316 and PAN6611 were the top 5 best and stable hybrids across the sites, respectively. These results specify that these hybrids performed better than the standard check, indicating that these hybrids need to be advanced in future breeding programmes and they should be evaluated in multilocation trials across South Africa. There are other authors who have reported on cultivar superiority and stability across different environments. These results are in accordance with Deitos et al. (2006) who reported that two cultivars were superior across three locations. Scapim et al. (2000) also reported that one cultivar was most productive in the different environments assessed, therefore was selected as the most stable. Ombakho et al. (2007) also reported that four hybrids were stable than others under the studied environments. Hence, these results are in accordance with the current study since four developmental hybrids were also stable across different environments.

5.5 CONCLUSION

The objectives of this study were to determine cultivar superiority of testcrosses among recombinant maize inbred lines and to select the best cultivar within four different environments. The findings provide sufficient evidence that there is variation in the performance of high yielding hybrids (top 25) within all the sites. Hybrid 10MAK10-1/N3 was selected as the best hybrid at Ukulinga in terms of grain yield, relative yield and economic traits. Hybrid T17/L83 was the best hybrid at Cedara in terms of grain yield and relative yield; however, it displayed poor performance in terms of economic traits and thus

T11/L102 was selected as the most balanced hybrid with respect to grain yield, relative yield and economic traits. Hybrid T3/L48 was also identified as the best hybrid at Dundee with respect to grain yield, relative yield and prolificacy. Finally, at Potchefstroom PAN6611 was identified as the best hybrid in terms of grain yield and relative yield followed by developmental hybrid T1/L28; however, developmental hybrid T1/L28 was the best in terms of earliness, prolificacy and ear aspect.

The overall hybrid distribution results revealed that at least five hybrids fitted in the desired matrix of grain yield by the economic traits in each environment which provides ample opportunity for selection of hybrids which will proceed to the advanced multilocation trials. While the results demonstrate the observation of significant breeding progress in all the target environments, there is still some opportunity for breeding to enhance yield of the new hybrids in western region.

Stability and cultivar superiority data across the sites revealed that four developmental hybrids were identified as best hybrids and they performed better than the standard check, they will thus be advanced in the multilocation breeding trials across South Africa in the next breeding programme.

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6.1 Introduction

Productivity of maize hybrids is compromised by stress caused by drought among other factors. However, stress tolerance can be enhanced by improving the adaptive traits in hybrids. In western South Africa, drought is prevalent while temperatures go down quickly towards end of the season as winter approaches. Therefore, appropriate hybrids design should combine grain yield with the following desirable traits: low grain moisture (earliness), prolificacy and lower ear aspect score. This chapter is a summary of the findings obtained in the whole study. The chapter also summarises the recommendations and suggests the way forward in studying genetic variation and character associations among adaptive traits in a recombinant maize inbred line population. The main objective of the study was to determine genetic variation for adaptive traits in a recombinant inbred line (RIL) population.

The specific objectives of the study were to:

- I. Determine combining ability of subtropical recombinant maize inbred lines with tropical testers;
- II. Determine genetic variation and associations among adaptive traits in hybrids involving maize recombinant inbred lines;
- III. Determine cultivar superiority of testcrosses involving recombinant maize inbred lines.

This was achieved through evaluation of hybrids between the 118 RILs and 9 tropical testers across four sites, representing at least two major environments, west and east maize belts in South Africa. Since this was a rapid screening of both the RILs and testers, an augmented experiment laid out as alpha lattice design was effective at confirming variation. The results would be crucial in defining the future course of the breeding programme. Therefore the major findings and implications for breeding, challenges and opportunities are emphasised.

6.2 Summary of the major findings and implications for breeding

6.2.1 Combining ability and genetic effects

The study confirms the presence of both additive and non-additive gene action; however, non-additive gene action had a competitive edge over additive gene action. Significant associations between yield and its components were observed where plant height was the most important component for grain yield.

- GCA effects due to the RILs played a non-significant ($p > 0.05$) role in determining grain yield, grain moisture and anthesis date, while they were important for the other traits, such as conferring ear prolificacy and desired short plant stature in hybrids with tropical testers.
- The tester main effects were significant for all the traits except number of ears per plant and plant height, indicating the kind of traits which compromise their adaptation to South African environments which are represented by these sites.
- The L1, 24 and 28 were the best general combiners for grain yield and prolificacy, whereas tester T11 was the best general combiner for grain yield, prolificacy, and plant and ear height, qualifying them as the most adapted lines with high utility in the programme.
- Gene action results revealed that all the traits were controlled by both additive and non-additive gene action, where additive gene action had the most contribution to the traits. Cross L114 x T12 had a significant and positive SCA effect for grain yield which is based on dominance gene action because the two parents exhibited the non-desired GCA effects. Many other crosses showed a similar trend. This indicates that although small the non-additive portion of the variance should not be ignored in designing hybrids.
- The correlation between yield and its traits was significant for prolificacy, grain moisture, ear height, plant height and anthesis date, indicating that indirect selection can be employed to enhance yield in South Africa by breeding for these particular adaptive traits.

Plant height had the highest direct and indirect effect on grain yield, indicating that this trait plays a major role in grain yield behaviour; therefore it can be both directly and indirectly manipulated for grain yield enhancement.

6.2.2 Genetic variation and associations among adaptive traits

The study confirms existence of ample genetic variation among the RILs, and significant associations among the traits which can be exploited:

- Phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the traits across all the four environments, indicating that variations in environmental conditions were more important than genetic factors in hybrid performance and these environments better represented different environmental domains.
- All the traits displayed high heritability especially at Potchefstroom and Cedara except anthesis which was highly heritable at Ukulinga, indicating that direct selection for the adaptive traits can be pursued to identify suitable hybrids.
- The genetic advance for grain yield was the highest at Cedara followed by Potchefstroom, Dundee and Ukulinga, but in general the range of 21 to 29% across the sites indicates that significant progress has been realised in finding new hybrids with enhanced adaptation ability in South Africa.
- The hybrids exhibited different patterns of variations for all the traits. The distribution of hybrids was continuous for all the traits in all the sites except anthesis date and plant height at Potchefstroom, indicating that many genes were involved in conferring the traits suggesting that yield and the complimentary adaptive traits can be enhanced by targeting the desired QTLs, in future studies.
- There were significant correlations between grain yield and its adaptive traits, and among adaptive traits. Ear length had the highest direct effect on grain yield at Ukulinga; number of ears per plant had the highest direct effect on grain yield at Cedara and Potchefstroom; whereas plant height had the highest direct effect on grain yield at Dundee, indicating that direct selection for ear size, prolificacy and plant height can be effective for improving yield in the target environments.

- Grain yield was least affected by indirect factors in all the sites except Ukulinga, where anthesis date had the highest indirect effect on grain yield through silking date followed by plant height through leaf area, suggesting that in general indirect selection of secondary traits will not be effective to enhance yield of the hybrids.

6.2.3 Cultivar superiority

The following hybrids were outstanding for yield and economic traits qualifying them as candidates for advanced trials in multilocation trials throughout the country:

- Hybrid 10MAK10-1/N3 was the best hybrid at Ukulinga in terms of grain yield, relative yield and economic traits.
- Hybrid T17/L83 was the best hybrid at Cedara in terms of grain yield and relative yield; however, it displayed poor performance in terms of economic traits and thus T11/L102 was selected as the most balanced hybrid with respect to grain yield, relative yield and economic traits.
- Hybrid T3/L48 was identified as the best hybrid at Dundee with respect to grain yield, relative yield and prolificacy.
- At Potchefstroom PAN6611 was identified as the best hybrid in terms of grain yield and relative yield followed by developmental hybrid T1/L28; however, developmental hybrid T1/L28 was the best in terms of earliness, prolificacy and ear aspect.
- Hybrids 11C2340, 11C2234, 11C2252, 11C2316 and PAN6611 were identified as five most superior and stable hybrids across the sites, respectively.

The results also reveal that there is still variation among the best yielding genotypes that fitted in the desired quadrant of yield plotted against each of the desired complimentary traits:

- At Ukulinga the results showed that five genotypes were both high yielding and prolific; eight genotypes produced high grain yield and low grain moisture content; and nine genotypes produced lower ear aspect and higher grain yield.

- At Cedara seven genotypes produced high grain yield and higher number of ears per plant; six genotypes produced high grain yield and lower grain moisture; and eleven genotypes produced lower ear aspect and high grain yield.
- At Dundee nine genotypes had high grain yield and number of ears per plant.
- At Potchefstroom ten genotypes produced higher grain yield and number of ears per plant; five genotypes had high grain yield and lower grain moisture; and six genotypes had higher grain yield and lower ear aspect.

6.3 General Implications and the way forward

The following implications and future directions were identified:

- In the future breeding programmes there is a need to cross all the elite lines and testers which showed the best combining ability for grain yield and certain desirable secondary traits for possible development of commercial hybrids.
- It is quite essential to further evaluate and improve all the crosses which displayed excellent specific combining ability. This would be done by testing the hybrids in at least 8 to 10 environments across South Africa.
- There is a necessity to increase the frequency of genotypes planted and evaluated at Potchefstroom since all the traits exhibited high heritability at Potchefstroom, meaning that results obtained at this site are most reliable, and very effective for discerning the RILs with desired traits.
- The amount of traits evaluated at Dundee should be amplified since there were few traits evaluated under this environment, so that it can be easy to determine proper hybrid performance. However, ways to overcome the logistical challenges of evaluating hybrids at this site should be found. This includes training some support staff who reside in the area to collect data such as flowering notes.
- There is also a need to evaluate all the hybrids which were selected as best in each environment across a number of environments to determine their stability across a number of environments.
- In future all the hybrids should be available across all the study sites including the local checks to ease the hybrid evaluation process. This will be made possible by

focusing on the most promising 25 hybrids that were identified in each of the four environments.

6.4 General conclusion

The main objective of the study was to determine genetic variation for adaptive traits and establish the associations between these traits and grain yield in a recombinant maize inbred line (RIL) population. The completed research was successful. The study confirms observation of significant variation among the RILs as they interacted differently with the 9 tropical testers. Even among the top 25 selections of RILs in each environment there was still variation for combinations of the desired traits. Significant associations among yield and the other economic and adaptive traits were observed with implications for breeding strategy. Above all the significant variation gives a large scope for future breeding of new unique products.