Genetic Effects and Associations between Grain Yield Potential, Stress Tolerance and Yield Stability in Southern African Maize (*Zea mays* L.) Base Germplasm

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Abstract

Maize (*Zea mays* L.) is the principal crop of Southern Africa but production is threatened by gray leaf spot (*Cercospora zea-maydis* L.) and phaeosphaeria leaf spot (*Phaeosphaeria maydis* L.) diseases, drought and the use of unadapted cultivars, among other constraints. There are few studies of gray leaf spot (GLS) and *Phaeosphaeria* leaf spot (PLS) resistance, drought tolerance, yield stability and maize cultivar preferences in Southern Africa. The objective of this study was to: a) determine farmers' preferences for cultivars; b) investigate the gene action and heritability for resistance to GLS and PLS, and drought tolerance; and c) evaluate yield stability and its relationship with high yield potential in Southern African maize germplasm. The study was conducted in South Africa and Zimbabwe during 2003 to 2004.

A participatory rural appraisal (PRA) established that farmers preferred old hybrids of the 1970s because they had better tolerance to drought stress. Farmers also preferred their local landrace because of its flintier grain and better taste than the hybrids. The major prevailing constraints that influenced farmers' preferences were lack of appropriate cultivars that fit into the ultra short seasons, drought and low soil fertility. Thus they preferred cultivars that combine high yield potential, early maturity, and drought tolerance in all areas. However, those in relatively wet areas preferred cultivars with tolerance to low soil fertility, and weevil resistance, among other traits.

A genetic analysis of 72 hybrids from a North Carolina Design II mating revealed significant differences for GLS and PLS resistance, and drought tolerance. General combining ability (GCA) effects accounted for 86% of genetic variation for GLS and 90% for PLS resistance indicating that additive effects were more important than non-additive gene action in controlling these traits. Some crosses between susceptible and resistant inbreds had high resistance to GLS suggesting the importance of dominance gene action in controlling GLS resistance. Resistance to GLS and PLS was highly heritable (62 to 73%) indicating that resistance could be improved by selection. Also large GCA effects for yield (72%), number of ears per plant (77%), and anthesis-silking interval (ASI) (77%) under drought stress indicated that predominantly additive effects controlled hybrid performance under drought conditions. Although heritability for yield declined from 60% under optimum to 19% under drought conditions, heritability for ASI ranged from 32 to 49% under moisture stress. High heritability for ASI suggested that yield could be improved through selection for short ASI, which is positively correlated with high yield potential under drought stress.

The stability analyses of the hybrids over 10 environments indicated that 86% had average stability; 8% had below average stability and were adapted to favourable environments; and 6% displayed above average stability and were specifically adapted to drought stress environments. Grain yield potential and yield stability were positively correlated.

In sum, the study indicated that farmers' preferences would be greatly influenced by the major prevailing constraints. It also identified adequate genetic variation for stress tolerance, yield potential and yield stability in Southern African maize base germplasm, without negative associations among them, suggesting that cultivars combining high yield potential, high stress tolerance and yield stability would be obtainable.

Declaration

The thesis study was carried out in the African Centre for Crop Improvement (ACCI), in the School of Biochemistry, Genetics, Microbiology and Plant Pathology, University of KwaZulu-Natal, Pietermaritzburg Campus, under the supervision of Professor P. Tongoona, Professor. W. de Milliano and Professor M. D. Laing.

Research presented in this thesis represents original work by the author and has not been otherwise submitted in any form for degree or diploma to any university. Where use has been made of the work of others it is duly acknowledged in the text.

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Acknowledgements

Dedication

To my father, Tumani (1936 to 1997), who lies in the cold soils of Chimanimani.
His counsel and vision remain my greatest inspiration to go for the stars.

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List of Abbreviations

Abbreviation

ART	Agricultural Research Trust
ASI	Anthesis to silking interval
CED	Cedara
CIMMYT	International Maize and Wheat Improvement Center
CSI	Cultivar Superiority Index
DMP	Days to 50% anthesis or mid pollination
DMS	Days to 50% silk emergence
EPP	Number of ears per plant
GCA	General combining ability
GLS	Gray leaf spot
IPCA	Interaction Principal Component Analysis
KRC	Kadoma Research Centre
LR	Leaf roll
Masl	Meters above sea level
PLS	Phaeosphaeria leaf spot
PRA	Participatory Rural Appraisal
RARS	Rattray Arnold Research Station
SCA	Specific combining ability
ST	Stapleford
SV	Save Valley

List of Tables

1.   The Importance of Maize in Southern Africa

Maize is the principal crop of Southern Africa. This sub-region consists of Angola, Botswana, Lesotho, Malawi, Mozambique, Namibia, South Africa, Swaziland, Zambia and Zimbabwe. Recent statistics show that 16 countries with the highest maize grain consumption in the world are in sub-Saharan Africa (Banziger and Diallo, 2002). Indeed, the per capita consumption within Southern Africa is even higher than that for sub-Saharan Africa as a whole. Banziger and Diallo (2002) reported that maize contributed 50% of calories in Southern Africa, compared to 30% in East Africa and 15% in West and Central Africa.

Maize is planted on 62 to 98% of the land allocated to cereals in Southern Africa (Table 1). Consumption of maize is particularly high in Lesotho, Malawi, South Africa, Zambia and Zimbabwe (Table 1). Maize thus plays a crucial role in feeding Southern Africans and sustaining their livelihoods. Low production levels of maize have serious implications for millions of Southern Africans.

2.   Global Disparities and Low Production of Maize in Sub-Saharan Africa

There are global anomalies in the production of maize. Globally maize commands a large share of farming area; it is produced on 44 million (M) ha in developed, and 96 M ha in developing countries (Pingali and Pandey, 2001). Despite this distribution of area, yield in developed countries is 8 t/ha, but slightly less than 3 t/ha in developing countries (Pingali and Pandey, 2001).

Amongst the developing countries themselves, there are further disparities. Third world production is dominated by four countries, namely China (26 M ha), Brazil (12 M ha), Mexico (7.5 M ha) and India (6 M ha), which produce 53% of production in developing countries (Pingali and Pandey, 2001). In sub-Saharan Africa, although the maize production area (22 M ha) is similar to that of China, these countries combined produced only 27 M tons in 2004 (FAOSTAT, 2004). The average yield of 1.2 t/ha in sub-Saharan Africa (FAOSTAT, 2004) is far below the average of 3 t/ha for the rest of developing countries.

Within the Southern African region there are further disparities in production (Table 1). South Africa contributes the bulk of grain in the region because production is by large-scale commercial farmers. In the other countries, small-scale or resource-poor farmers largely produce the crop. Mozambique and Angola registered the highest growth in production, while Zambia, Zimbabwe and South Africa had negative growth rates.

Table 0.1: Maize production data for countries in Southern Africa

Country	Consumption Per Capita (kg/yr)[*]	Area of Cereals [*] (%)	Area (x 1000 ha)	Yield (t/ha)	Production (X 1000 t)	Production Growth[*] (%/yr)
Angola	36	76	980	0.5	510	6.3
Botswana	---	---	84	0.1	10	---
Lesotho	135	71	180	0.8	150	0.7
Malawi	148	89	1550	1.1	1733	3.4
Mozambique	53	62	1300	1.0	1248	14.5
Namibia	---	---	23	1.4	33	--
South Africa	101	74		2.3	8517	-0.5
Swaziland	60	98	60	1.2	70	0.8
Zambia	140	78	750	1.5	1161	-6.1
Zimbabwe	122	75	1400	0.7	1000	-0.7

Source: FAOSTAT (2004); [*]Pingali and Pandey (2001)

3.   Maize Production Constraints in Southern Africa

Possible factors that have been advanced to explain the disparities in average yield between developed and developing countries are the different environments and the available technologies. Thus, most developed countries produce maize in temperate climates, but only China and Argentina among the developing countries grow the crop under temperate conditions. In tropical sub-Saharan Africa, small-scale farmers dominate production of maize under highly stressful conditions of disease, low soil fertility and drought, and with limited access to the essential inputs (Banziger and de Meyer, 2002). In most cases, these farmers have either little or limited access to improved technologies. For example, less than 50% of these small-scale farmers grow the new improved cultivars (Banziger and Diallo, 2002).

Maize yield potential for sub-Saharan Africa varies according to mega-environment but the yield gap, which is the gap between yield potential and actual yield, is consistently high across all three mega- environments (Fig. 1). Generally, this gap between yield potential and farmers' actual yield exceeds 50%. This suggests that there are, in fact, various stresses, which constrain production of even improved

cultivars across all three mega-environments regardless of the suitability of climate for maize production. (Tollenaar, in his 2002 study, defines stress as any factor resulting in yield reduction.)
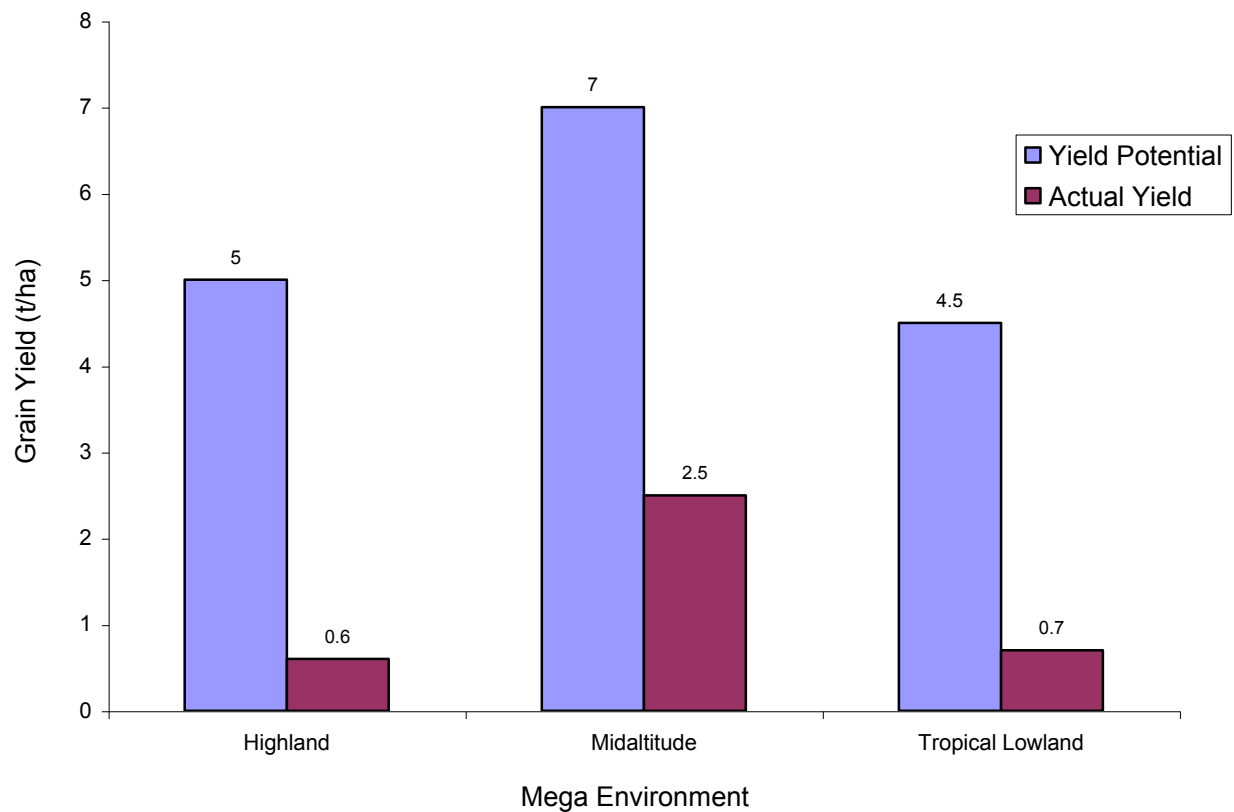
Fig. 1: Comparison of yield potential and actual yield in sub-Saharan Africa
(Data: Pingali and Pandey, 2001)

Pingali and Pandey (2001) reported dominant production constraints, identified by the International Maize and Wheat Improvement Centre (CIMMYT), across the three mega-environments of sub-Saharan Africa. These constraints included low soil fertility, drought, gray leaf spot disease and limited technology options (Table 0.2).

Low and declining soil fertility is the highest ranked constraint across all three mega-environments. Although low soil fertility is a serious threat to regional food security, it is a static factor, hence farmers would know the condition of their soil at the time of planting and could take this into account.   However, low soil fertility, especially low N, remains one of the major production constraints due to the limited access to fertiliser in developing countries. A focus on breeding for drought tolerance has been reported to have spillover effects to low soil N problems. Edmeades *et al.* (1997)

reported that cultivars selected for drought tolerance also showed improved yields under low N conditions.

The other, less predictable constraints of drought, disease and pests pose additional threats to regional food security. Drought and diseases such as gray leaf spot (GLS) and phaeosphaeria leaf spot (PLS) are weather-dependent, hence their unpredictability. Small-scale farmers cannot afford or have limited access to irrigation facilities and pesticides to manage drought and diseases, respectively.

Table 0.2: Dominant production constraints in sub Saharan Africa

Rank	Highland/ Transitional	Mid-Altitude/Subtropical	Tropical Lowland
1	Low and declining soil fertility	Low and declining soil fertility	Low and declining soil fertility
2	Limited technology options	Gray leaf spot	Drought
3	*Turcicum* blight	Maize streak virus	*Striga* sp.
4	Rust	Grain Weevils	Maize streak virus
5		Borers (*Chilo, Sesamia* spp.)	Maize borers
6		Drought	

Source: Pingali and Pandey (2001)

Frequent drought that reduces production is common in Southern Africa. The weather pattern is variable and highly favourable seasons are often followed by unfavourable years which impact on economic growth and food security. For example, Richardson (2003) reported that the gross domestic product (GDP) of Zimbabwe showed a positive correlation (r = 0.65) with the amount of rainfall. Banziger and Diallo (2001) reported a positive relationship between rainfall and average yield across the subcontinent. The distribution of rainfall varies even within seasons in all production areas (Olver, 1998). Campos *et al*. (2004) reported that available soil moisture could also vary considerably even within the same field. Fig. 2 presents the fluctuation of grain production which is attributable to rainfall variation, among other factors, in Malawi, Zambia and Zimbabwe from 1961 to 2003. Thus, severe droughts have periodically reduced grain production because more than 93% of the crops are not irrigated (Banziger and Diallo, 2002; Pingali and Pandey, 2001). Campos *et al*. (2004) therefore suggested that appropriate cultivars for release should carry base-line tolerance to drought regardless of the area of their deployment.

Drought causes maximum damage and yield reduction around flowering (Cakir, 2004). Farmers can replant if drought occurs at the seedling stage and obtain reduced yield if drought occurs late in the season. Drought at flowering can only be mitigated by irrigation, which unfortunately is beyond the reach of many smallholder farmers. Edmeades *et al.* (1995) reported that pollination of late emerging silks under drought leads to fertilisation, but grain development is arrested, resulting in patchy cobs or complete barrenness. Breeding for drought stress tolerance in maize would thus contribute to alleviating substantial drought induced loss; it should be targeted at flowering and would entail selecting cultivars with a short anthesis-to-silking interval.
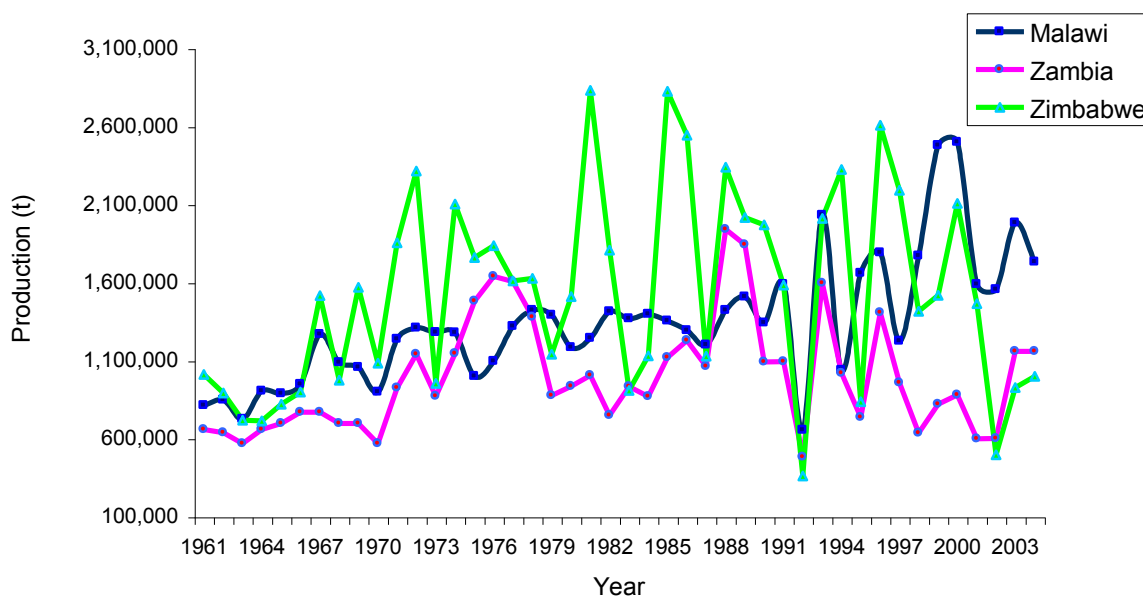
Fig. 2:   Variation of Grain Production in 3 Countries (Data: FAOSTAT, 2004)

Among the biotic constraints, GLS, caused by the fungus *Cercospora zeae-maydis*, is a major disease worldwide. Grain yield losses of ±30%, attributable to GLS, have been reported in many countries (Pingali and Pandey, 2001). Since the 1990s, GLS has spread and is now endemic throughout sub-Saharan Africa (Menkir and Ayodele, 2005). Ward *et al.* (1997) estimated yield losses at 63% in South Africa, indicating that the disease can be devastating if susceptible cultivars are grown.

Phaeosphaeria leaf spot, caused by the fungus *Phaeosphaeria maydis,* is another disease with the potential to threaten regional food security. Although not mentioned among the dominant constraints, the incidence of PLS has been on the increase (Vivek *et al.*, 2001). Carson (1999) reported a high incidence of PLS in South Africa and Zimbabwe among other countries. Grain yield loss due to PLS alone has not yet

been quantified in this region but substantial losses have been reported in Brazil. Paccola-Meirelles (2001) reported that PLS inflicted heavy yield losses of 60% in susceptible cultivars in Brazil. Like GLS, phaeosphaeria causes yield losses through accelerated leaf senescence, a reduced plant life cycle, and decreased grain size and weight in susceptible cultivars. Grain yield losses would be devastating if PLS were to occur together with GLS before grain filling.

In order to address the problem of food insecurity in the Southern African region, there is' therefore, an urgent need to start breeding for maize resistant to the major and unpredictable stresses of drought and disease, in particular GLS and PLS.

A further consideration for the maize breeder in Southern Africa is the issue of yield stability. The highly variable rainfall pattern and the unevenness of the production environment on the subcontinent give rise to complex cultivar x environment (G x E) interactions. Cultivar x environment interaction has been defined as the differential response of cultivars across environments (Crossa, 1990). Breeding progress is delayed by G x E, due to changes in cultivar ranking in different environments. In other words, there are different compositions of the selected and rejected cultivars in different environments (Crossa *et al*., 1995). Furthermore, Campos *et al*. (2004) reported that there are cultivar x timing of stress interactions in stressful environments.

Small-scale farmers in Southern Africa do not have adequate means to modify or condition the production environment, therefore selection for yield stability is desirable. Stable cultivars have reduced interaction with environments. Tollenaar and Lee (2002) defined yield stability as the ability of a cultivar to maintain relative yield across environments. An appropriate cultivar exploits resources such as high soil moisture and high fertility that are available in favourable environments and maintains acceptable yield in low the input environments (Finlay and Wilkinson, 1963). Selection of cultivars for high yield stability should be a high priority for the variable environments in Southern Africa.

4.   Farmer Preferences in Maize Breeding

Farmer preferences have emerged as a major factor in the improvement of maize yield in smallholder farming. As reported by Banziger and Cooper (2001), superior cultivars have not always been adopted, even where available, because they do not meet farmers' preferences. This phenomenon may contribute to the significantly

large yield gap (Fig. 1) in developing countries – smallholder farmers reject available improved seed.

Apart from high yield and disease resistance, breeders may not know farmers' complex requirements. Small-scale farmers have some specially preferred traits, which may not be considered by breeding institutions. According to Banziger *et al*. (2004), regional programmes have a tendency to focus their breeding goals on the requirements of the commercial farming sector. Perhaps, the small-scale farmers might not have interest in growing some hybrids that have not been developed to meet their specific requirements (Kamara, 1996, as cited by Diallo and Banziger, 2001). Effective cultivar breeding for deployment in marginal areas should be based on the identified constraints and specific preferences for small-scale farmers.

5.   Summing Up of Rationale for Research Focus

Grain production is highly variable in Southern African countries, which is a reflection of the annual variation of precipitation among other constraints. It is likely that cultivars would be subjected to GLS and PLS diseases during wet seasons, which provide favourable conditions for epidemic development. In other words, the incidence of GLS and PLS is highly weather-dependent, with the result that disease becomes the major constraint in a favourably wet season. The same cultivars would be challenged by drought in the same area if the following season were unfavourable. Possibly cultivars could be challenged by both disease and drought stress in one season. This is because the distribution of rainfall varies even within seasons in all production areas. Plant available soil moisture could also vary considerably even within the same field. Due to these highly variable conditions in the production environment, breeding for cultivars with base line tolerance to drought and resistance to GLS and PLS is suggested. Cultivars should also be bred for high yield stability so that farmers would not suffer yield penalty in the event of a favourable season. In addition, cultivar design should include specific preferences for small-scale farmers in marginal areas.

6.   Research Hypotheses

In order to develop effective breeding strategies for inherent baseline tolerance to drought, GLS, and PLS, without sacrificing yield, there is a need to have adequate knowledge of the following factors: a) farmers' preferences; b) the level of genetic variation for resistance; c) gene action controlling yield and tolerance to GLS, PLS and drought; d) heritability of resistance, grain yield and its associated traits; and e)

the relationship between yield stability, grain yield potential, and stress tolerance to GLS, PLS, and drought in Southern African adapted base germplasm.

This study was undertaken to test the following hypotheses:

a) Small-scale farmers in Southern Africa recognise the key production constraints peculiar to their areas and have specific preferences for stress tolerant maize cultivars,

b) There is adequate genetic variation for grain yield and its associated traits which is highly heritable and is exploitable in a breeding programme to generate drought tolerant materials.

c) There is sufficient genetic variation and high levels of resistance to GLS and PLS which are highly heritable and are exploitable in a breeding programme to generate disease resistant materials.

d) There are significant positive relationships between stress tolerance, grain yield potential, and yield stability in Southern African adapted maize germplasm.

7. Specific Objectives and Structure of Thesis

The specific objectives of this study were achieved in the various chapters as follows:

Objective	Chapter
1. Review relevant literature on grain yield, stability, GLS, PLS and drought stress tolerance.	Chapter 1
2. Investigate farmer perceptions on maize cultivars and their implications for breeding, using the marginal eastern-belt of Zimbabwe as a case study.	Chapter 2
3. Determine (i) levels of resistance, (ii) heritability, (iii) gene action conditioning resistance to gray leaf spot, and (iv) the relationship between gray leaf spot resistance and grain yield in a selected set of Southern African maize.	Chapter 3
4. Determine (i) levels of resistance, (ii) heritability, (iii) gene action conditioning resistance to phaeosphaeria leaf spot, and (iv) the relationship between PLS resistance and grain yield in a selected set of Southern African maize.	Chapter 4
5. Establish levels of (i) drought stress tolerance, (ii) gene action and combining ability of inbred lines, (iii) heritability, and (iv) correlations between grain yield and secondary traits under drought stress.	Chapter 5
6. (i) Evaluate level of stability of grain yield and (ii) determine the relationship between yield stability and grain yield potential in a selected, but representative sample of Southern African base germplasm.	Chapter 6
7. Review and conclude the completed research.	Chapter 7

This thesis is presented in a composite form, with the Chapters 2 to 6 intended for publication. For this reason, there may be overlapping of content and references.

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Chapter 1: Literature Review

1.1 Introduction

This chapter provides a context for the research by a) reviewing theory relevant to maize germplasm (heterotic) groups, gene action, grain yield, stress tolerance, and yield stability, b) reviewing literature on grain yield, gray leaf spot and phaeosphaeria leaf spot diseases and the drought problem, and c) defining key technical terms of the study. Gray leaf spot and drought represent the major biotic and abiotic constraints in Southern Africa, whereas phaeosphaeria leaf spot disease has very little published information. Thus, this chapter creates a frame of reference for the study.

1.2 Maize Heterotic Groups

Knowledge of genetic diversity of maize germplasm is important in designing hybrids. Expression of heterosis depends on the differences in the gene frequency of the parent materials (lines or cultivars) that are used to make crosses. The best hybrid vigour or the highest heterotic responses are obtainable when crosses are made between parents originating from genetically different populations (Hallauer and Miranda, 1988). Hybrid oriented breeding programmes use different heterotic groups for specific regions, but there are some heterotic patterns that are used across regions depending on their adaptation – whether they have specific or wide adaptation (Mickelson *et al*., 2001). There are at least nine main heterotic groups of elite inbred lines that are used by the maize breeding programmes in Eastern and Southern Africa (Table 1.1). The SC, N3 and K64R derived lines are key components of hybrids in national breeding programmes (Olver, 1998; Mickelson *et al*., 2001). The K64R lines and their derivatives have been widely used to constitute early maturing hybrids in Zimbabwe (Olver, 1998). The rest of the inbred lines are mainly used in South African hybrid-oriented programmes (Cowie, 1998). The full descriptions of these groups have been presented in detail (Gevers and Whythe, 1987; Gevers and Lake, 1998; Olver, 1998). Almost all breeding programmes in East and Southern Africa use lines from CIMMYT. The CIMMYT classification is broader based than the regional categorisation (CIMMYT, 2001). In the current study, experimental hybrids were constituted between lines that were drawn from these heterotic groups (Chapters 3 to 6), because they represent regional base germplasm.

Table 1.1. Main heterotic groups of maize inbred lines used in Southern Africa

Heterotic Group	Population of derivation	Examples of Public Lines	Reference
SC	Southern Cross	SC5522	Mickelson *et al.* (2001)
N3	Salisbury White	N3-2-3-3	Mickelson *et al.* (2001)
K	K64R/M162W	K64R, M162W	Mickelson *et al.* (2001)
P	Natal Potchefstroom Pearl Elite Selection (NPP ES)	NAW5867	Gevers and Whythe (1987) Olver (1998)
I	NYHT/TY	A26, I137TN	Gevers and Whythe (1987)
M	21A2.Jellicorse	M37W	Gevers and Whythe (1987)
F	F2934T/Teko Yellow	F2834T	Gevers and Whythe (1987)
CIMMYT- A	Tuxpeno, Kitale, BSSS, N3 (More Dent Type)	CML442, CML202	CIMMYT, 2001
CIMMYT- B	ETO, Ecuador 573, Lancaster, SC (More Flint Type)	CML444, CML395	CIMMYT, 2001

1.3   Gene Action and Its Implication

In order to develop effective crop breeding strategies for inherent baseline tolerance to the major stresses without sacrificing yield potential, there is a need to understand the nature of gene action operating for grain yield and the associated traits under stress-prone tropical environments in Southern Africa.

1.3.1 Defining Gene Action

Genes, located on chromosomes, represent the basic units of inheritance, and control the expression of characters, individually or in combinations. Gene action is the way genes express themselves. Gene action is studied using the Mendelian and the biometrical genetic approach. In Mendelian genetics, the dominant gene action refers to the expression of a character that appears in the F_1 generation; but recessive genes are not expressed in the presence of dominance in the F_1, but their traits re-appear in the segregating F_2 generation (Welsh, 1981).

In quantitative genetics, genetic components are divided into additive and dominance variance and epistasis (Robinson *et al*., 1949; Falconer, 1981). In the presence of additive gene action, characters of heterozygotes in the F_2 generations are the intermediate of the two parents, because additive variation is associated with the average effects of the particular alleles (Falconer, 1981). The additive portion reflects the degree to which progenies are likely to resemble their parents, which is

reflected in the narrow sense heritability. Knowledge of heritability indicates to the breeder the possibility to which genetic improvement is possible through selection.

Non-additive gene action is observed when the additive model cannot adequately explain the variation (Falconer, 1981). According to Robinson *et al*. (1949), the size of dominance relative to the additive variance indicates the degree of dominance. Thus, levels of dominance in the progeny display a range from partial to over-dominance in relation to the mean of their parents (Table 1.2). Sharma (1994) defined average performance of the parents as the mid-parental value (*m*).

Table 1.2. Classes of dominance gene action

Type of Dominance	Description
Partial dominance	Heterozygote has a value that is closer to one parent
Positive partial dominance	Performance of the heterozygote lies between *m* and the value of the superior parent
Negative partial dominance	Performance of a heterozygote lies between *m* and the value of the inferior parent.
Positive complete dominance	Performance of the heterozygote equals that of the superior parent
Negative complete dominance	Performance of the heterozygote equals that of the inferior parent.
Positive over-dominance	Performance of the heterozygote exceeds that of the superior parent
Negative over-dominance	Performance of the heterozygote is less than that of the superior parent

Source: Hallauer and Miranda (1988); Sharma (1994)

Epistatic gene action occurs when variation cannot be explained on the basis of the additive-dominance model alone. Epistasis is the interaction of alleles at different loci and there are three types of epistasis (Table 1.3).

Table 1.3. Types of epistatic gene interaction (Falconer, 1981).

Type of Epistasis	Description
Additive x Additive	Interaction occurs when two or more genes, each showing additive action individually, interact with each other causing differential genotypic values as influenced by the genetic content of genes situated at other loci. Variation is created by the interaction of two genes, each acting additively.
Additive x Dominance	Interaction occurs when two or more loci, one showing additive and the other dominance action, interact.
Dominance x Dominance	Interaction occurs when two or more loci, each showing dominance action individually, interact.

1.3.2 Estimating Gene Action

Hallauer and Miranda (1988) reviewed the various mating designs and methods for estimating genetic variance in maize populations. These consist of analyses of bi-parental progenies, parent-offspring regression, diallel, and North Carolina (NC) I, II and III mating designs. Assumptions underlying these designs are as follows: (a) a random choice of parents for crossing; (b) parents randomly distributed; (c) absence of maternal effects; (d) diploid behaviour; (e) no multiple alleles; (f) linkage equilibrium of genes; and (g) no epistasis (Hallauer and Miranda, 1988; Dabholkar, 1992). These assumptions should be validated when interpreting results from genetic studies.

Biparental mating is the simplest design. Pairs of randomly selected plants from a population are crossed. The level of genetic variation is then determined by evaluating the crosses in replicated trials. The limitation of this design is that it does not determine the type of gene action, but only the level of genetic variation is determined (Hallauer and Miranda, 1988).

In parent-offspring regression, randomly selected plants from a population are crossed. Parent-offspring regression estimates heritability of traits by regressing the mean of the cross on the mean value of its parents (Hallauer and Miranda, 1988). As a result in this deisgn both parents and the crosses are evaluated. This design is mainly applied for ae quick estimate for the level of heritability of plant traits in a population.

A diallel mating entails making all possible single crosses among a group of parents. There are four types of diallel mating designs, depending on whether the design evaluates crosses, parents and reciprocals (Stuber, 1980; Hallauer and Miranda, 1988; Dabholkar, 1992). A complete diallel evaluates the variances due to the crosses, parents and reciprocal effects (Stuber, 1980). In a half diallel, only the crosses are evaluated. The third type of diallel evaluates the crosses and the reciprocal effects without the parental effects. In the fourth diallel type, the variances for the crosses and parents are determined without including the reciprocal effects. All the diallel types estimate variation due to the crosses; this is partitioned into sources due to general combining ability (GCA) and specific combining ability (SCA). So the differences between the diallels are based on whether parents or reciprocal effects are included in the model. The reciprocal crosses estimate the variation due

to maternal effects, which are expected for some traits. Practically, the design is most applicable with few parents (≤10), because use of many parents results in too many crosses to manage (Stuber, 1980; Hallauer and Miranda, 1988).

In a North Carolina I mating design (Comstock and Robinson, 1948), a group of female parents are crossed to a common male. This design can be used to produce a large number crosses that may be required for evaluation in breeding programmes. The NC I estimates GCA variance for the male and the female within male variances. The performance of female parents is compared within a particular male, and comparisons between the different males are also possible. It seems the limitations of this design of this design are that it does not estimate SCA and reciprocal effects.

The North Carolina II mating design (Comstock and Robinson, 1948) is a factorial experiment that measures the variance of male and female main effects and the male x female interaction effects. According to Hallauer and Miranda (1988), the male and female main effects, and the male x female interaction effects in a NC II mating design are equivalent to the GCA and the SCA effects in a diallel. The main difference between a diallel and NC II is that there are two independent estimates for the GCA effects in the NC II, which is an advantage of the NC II over the diallel. Two independent estimates of GCA allow determination of maternal effects and calculation of heritability based on the male variance, which is free from maternal effects. Another advantage is that the NC II can handle more parents and produce fewer crosses than the diallel. In the NC II, dominance variance can be determined directly from the male variance. An additional advantage of the NC II is that crossing of parents in sets can increase the sample size to be tested (Hallauer and Miranda, 1988).

In NC III mating design (Comstock and Robinson, 1948), an F_2 population is formed between two inbred parents. The F_2 plants are then backcrossed to the parents. This design estimates dominance and additive variances and estimates dominance levels. It can also be used to estimate the effects of linkage on the additive and dominance variances. The NC III has an advantage over the NC II because it can measure levels of dominance.

 In the current study, a NC II mating design (Comstock and Robinson, 1948; 1952) was used to form hybrids among 27 inbred lines that were evaluated for stress tolerance. The NC II was selected for the study because the objective was to

estimate GCA and SCA variances, which could not be generated by biparental, and regression methods. North Carolina III mating design was not used because the design involves two inbred parents only. However, a serious choice had to be made between using a diallel and NC design II.

A NC II mating design was chosen instead of the diallel in order to generate fewer crosses from more parents (> 10). This could be achieved in a NC II mating by dividing the parent inbred lines into sets and then forming the crosses among subsets, which effectively increased the sample size for the study. Use of a diallel design would have generated more crosses with the same number of parents, which are difficult to manage in trials. Compared to the diallel, the NC II mating design has two independent estimates for the GCA due to male and female parent sources. Although the diallel has the advantage of incorporating reciprocal effects in the model for checking maternal effects, the NC II mating can also estimate maternal effects by testing the differences between the male and female mean squares. As a result, heritability can be calculated using the male variance, which is free from the maternal effects. If present, these maternal effects would lead to the upward bias of the additive variance (Hallauer and Miranda, 1988).

1.4   Grain Yield and Stress Tolerance

1.4.1 Defining Yield and Stress Tolerance

Primarily breeding aims at developing cultivars that satisfy farmers' requirements. Evans and Fischer (1999) defined grain yield as the grain mass with specific moisture content. "Yield potential" is obtained when a cultivar is grown under non-limiting conditions and in an environment of its adaptation, or in the absence of stress. Therefore, stress tolerance is defined in relation to yield potential. Tollenaar (2002) defined stress tolerance as the ability of cultivars to mitigate the impact of stress. The difference between "yield potential" and "actual yield" reflects the level of stress tolerance of a cultivar. Thus, as the actual yield approaches yield potential, cultivars are regarded as relatively stress tolerant.

1.4.2 Progress in Improving Grain Yield Potential

Appreciable progress has been realised in improving grain yield, especially in temperate maize. Tollenaar and Lee (2002) have reported grain yield potential of 14.5 to 20.9 t/ha in the USA, compared with the actual yield of ±7 t/ha. Other studies

reported that the actual yield was only about a quarter of the yield potential for the USA (Tollenaar, 1983; Tollenaar, 2002). However, Duvick and Cassman (1999) reported attainable yield of 18 t/ha under irrigated conditions in Nebraska (USA). In tropical sub-Saharan Africa, Pingali and Pandey (2001) reported yield potential of 5 t/ha against actual yield of 0.5 t/ha in highland/transitional zones; 7 t/ha versus 2.5 t/ha in mid-altitude/subtropical zones; and 4.5 t/ha versus 0.7 t/ha in tropical lowland environments. In Eastern and Southern Africa, Banziger and Diallo (2002) reported actual yield of 1.3 t/ha for small-scale farmers and 4 to 14 t/ha for the researchers. It appears that conditions for obtaining a high yield are hard to achieve by researchers, let alone for the resource-constrained, small-scale farmers in marginal areas of Southern Africa. The wide gap between actual and yield potential indicates that there is still a huge opportunity for improving grain yield, especially in tropical sub-Saharan Africa.

1.4.3 Stress Tolerance as a Basis for Yield Improvement

Breeding for stress tolerance is not new. There is overwhelming evidence in support of the predominance of stress tolerance in explaining yield improvement in temperate maize. Duvick (1997) reported that improved grain yield potential of the best hybrids in Central Iowa was due to stress tolerance and high yield per plant. Tollenaar and Wu (1999) reported that improved stress tolerance was associated with lower plant-to-plant variability. Tollenaar *et al.* (1997) reported one case where new hybrids were even more competitive with weeds than the old hybrids. Superiority of hybrids under stress sharply contradicts the opinion that genotypic and phenotypic heterogeneity are positively correlated with yield stability. Tollenaar and Wu (1999) reported that single cross hybrids had better adaptation and stress tolerance than genetically variable open pollinated cultivars and double cross hybrids. Previously, Troyer (1996) reported that the trend of maize evolution in the USA, beginning with open pollinated varieties (OPVs) in the 1930 era, and followed by double cross (1930-1960s) and single cross hybrids (late 1960s), was associated with improved yield and adaptation. Arguably, the more than 20% yield improvement could not be attributed to heterosis alone (Duvick, 1992, 1997; Duvick and Farnham, 1997). However, an equal effort has not been applied to the development and improvement of OPVs in the USA as breeding emphasis of researchers seems to have shifted towards the hybrids after the 1960s era. Perhaps if they had applied an equal effort to research and development of OPVs, the grain yield potential and yield stability could be more comparable or even higher than that of the hybrids.

Studies by Tollenaar and Wu (1999) have suggested that there are common mechanisms for conferring tolerance to different forms of stress in temperate maize cultivars. This has also been reported in some tropical cultivars that had tolerance to both drought and low soil N (Banziger *et al.*, 2002; Lafitte and Edmeades, 1995), but such studies are still limited. High level of stress tolerance might have resulted in part from selection for improved grain yield stability in many multi-locational trials (Tollenaar and Lee, 2002; Tollenaar, 2002). Furthermore, cultivars have increasingly been tested under conditions representing commercial production (Tollenaar and Lee, 2002). Another explanation for the success in yield improvement is recycling of the best inbreds in pedigree breeding. Duvick (1997) reported that breeders used inbred lines derived from the best hybrids to develop new hybrids. It has also been argued that yield improvement was a result of improved efficient use of resources due to delayed leaf senescence or the "stay green" trait (Duvick, 1997; Tollenaar and Wu, 1999), which improved grain filling.

1.4.4 Gene Action Conditioning Grain Yield

Different types of gene action control grain yield and its associated traits. Betran and Hallauer (1996) reported that additive effects were more important than dominance for grain yield, lodging, and the flowering days in hybrids. Wolf *et al.* (2000) reported larger dominance than additive effects for grain yield in an F_2 of B73 x Mo17. Wolf *et al.* (2000) reported high level of dominance of 2.44 comparable to 1.28 that had been previously reported by Han and Hallauer (1989) for grain yield. At times the high levels of dominance are a result of an upward bias by linkage disequilibrium in F_2 single crosses of inbred lines.

There are different reports regarding contribution of epistasis in conditioning grain yield that has generally been reported to be negligible (Darrah and Hallauer, 1972; Eta-Ndu and Openshaw, 1999; Hinze and Lamkey, 2003; Lamkey *et al.*, 1995; Melchinger *et al.*, 1988; Wolf and Hallauer, 1997). However, Wolf and Hallauer (1997) reported significant epistasis effects for ear traits, days to flowering and grain yield. Wolf and Hallauer (1997) suggested that favourable epistasis could have contributed to heterosis in the highly adapted B73 x Mo17 hybrid. Favourable epistasis can be fixed through repeated selfing (Lamkey *et al.*, 1995; Wolf and Hallauer, 1997).

This review shows that additive, dominance and epistasis effects explained high yield in the widely adapted hybrids. However, such studies have been limited to temperate germplasm. A survey of literature revealed that little research has been conducted on gene action in African maize populations. Relevant information about inheritance should be generated from regional maize grown under sub-Saharan African environments. According to Falconer (1981) information from genetic studies is specific to the specific germplasm and the environments tested. Thus, information generated in temperate maize and temperate environments might not have direct application in Southern Africa. In the current study, diverse germplasm drawn from nine major heterotic groups, which are used by breeding programmes in Southern Africa were evaluated for gene action in Southern African environments.

1.5   Genotype x Environment Interaction

In addition to genotype and environments main effects, performance of cultivars is also determined by the genotype x environment interaction (G x E), which is the differential response of cultivars to environmental changes (Vargas *et al*., 2001). There are three common types of G x E interaction, namely cultivar x location interaction; cultivar x year interaction; and cultivar x location x year interaction effects (Crossa, 1990). These G x E interactions are explained by variation in weather between and within seasons and soil properties, among other factors. For example, Troyer (1996) reported that cultivar x year interaction was larger than cultivar x location interaction due to differing soil moisture availability at flowering. Crossover interaction is the G x E interaction that changes the rank order for performance of cultivars. At times G x E does not change the rank order except for absolute differences of cultivar performance in the different environments. Crossover interaction causes problems in crop breeding because it impedes selection progress due to changing composition of cultivars selected in different environments (Crossa *et al*., 1995; Cooper and Delacy, 1994).

1.6.1 Assessment of Grain Yield Stability

Stable cultivars have little interaction with environments (Tollenaar and Lee, 2002). Becker and Leon (1988) defined two types of stability, namely static or dynamic. In a static stability, cultivar yield does not change; but with dynamic stability cultivar yield changes in a predictable manner, and its stability is affected by the set of cultivars under evaluation (Becker and Leon, 1988; Tollenaar and Lee, 2002). Thus, static

stability is an absolute measure, while dynamic stability is a relative measure. In cultivar selection, the best cultivar should effectively exploit the high inputs under favourable conditions and display acceptable grain yield under relatively low input systems (Finlay and Wilkinson, 1963) suggesting that dynamic stability could be preferred. This dynamic concept of grain yield stability is measured by the regression analysis as described by Finlay and Wilkinson (1963) and is sometimes referred as the parametric statistic. Although it is widely used it some limitations that have been mentioned by many researchers (Section1.6.2), hence there are some alternative statistical methods for measuring stability.

Lin *et al.* (1986) reviewed the nonparametric statistics for evaluating G x E. These stability statistics are not influenced by the set of cultivars under evaluation. Lin *et al*. (1986) defined a stable cultivar as having a small variance and a similar deviation from the overall mean yield in all the environments. Lin and Binns (1988) also reported the cultivar superiority index, which they defined as the mean square of the differences between the cultivar's response and the maximum response in different environments. Thus, there are different methods for assessing stability, hence scientists can choose whether to use parametric or the nonparametric statistics (Section 1.6.2).

Grain yield stability is influenced by the genetics of the cultivar. Eberhart and Russell (1966) reported that the use of genetic mixtures rather than homogeneous cultivars reduced G x E interaction due to population buffering in a heterogeneous population. Recently, Lee *et al*. (2003) also reported that double cross hybrids had smaller G x E interaction, than single cross hybrids, which are more homogeneous. However, it is also possible that some single crosses could be more stable than the three-way and double cross hybrids (Eberhart and Russell, 1966). Grain yield stability can be improved through recurrent selection because it is heritable and largely controlled by additive gene action (Lee *et al.,* 2003). In addition, stable cultivars can be identified through multilocation trials in targeted environments (Troyer, 1996). The high grain yield potential and adaptation of Pioneer hybrids to the USA were obtained through extensive multilocation trials (Duvick, 1997; Evans and Fischer, 1999). It was, thus, found prudent in the current study to evaluate regionally important germplasm under disease and drought stress environments.

1.6.2 Limitations of the Regression Approach in Stability Analysis

Crossa (1990) reviewed the limitations of regression analysis of stability. Crossa (1990) reported that with few cultivars (less than 15) the mean of cultivars would not be independent of the marginal means of the environments. The regression analysis is not effective in the absence of a linear relationship between cultivar x environment interaction and the environmental means. Stability of a cultivar measured by regression analysis of a few and or extreme environments would not provide reliable information, due to the high levels of bias. In the same vein, stability of a cultivar depends on the set of cultivars evaluated; hence application of the results from a regression analysis is limited to the specific set of environments and cultivars evaluated. Alternatives to the regression analysis are several nonparametric statistics. Huhn (1990) reviewed the rank analyses used in studying G x E interactions. These statistics have some advantages over the regression analysis such as reduction of bias caused by outlying cultivars and they are easy to interpret. In addition, the assumptions about the distribution of data, homogeneity of variances and linearity are not required for rank analyses (Huhn, 1990). In the current study, both parametric and nonparametric statistics were used to estimate stability of 80 hybrids in 10 environments. Therefore, a hybrid was considered stable if it appeared stable in more than three stability statistics. Arguably, the large number of hybrids (80) and environments (10) could have reduced the effects of some of the limitations of the regression analysis.

1.7 Gray Leaf Spot Disease

In sub-Saharan Africa, the gray leaf spot (GLS) has become endemic throughout the humid areas in Western, Eastern and Southern Africa (Menkir and Ayodele, 2005; Caldwell, 2002). Gray leaf spot is caused by *Cercospora zeae-maydis* Tehon and Daniels, with two isolates that have been reported in the USA (Lipps *et al.*, 1998). These isolates vary in their aggressiveness but they do not have physiological specialisation into races (Carson, 1999). Only one isolate is found in Africa, which is similar to the one that is most prevalent in the USA (Lipps *et al.*, 1998). Each isolate was relatively uniform suggesting asexual reproduction (Lipps *et al.*, 1998), which minimises emergence of different races of GLS.

1.7.1 Disease Cycle, Epidemiology and Control

Severe GLS occurs in monoculture situations when the pathogen over-winters in maize debris on the soil. The disease development is highly weather dependent.

Conidiaspores are disseminated by wind or water and require 72 hours of $\geq$ 95% relative humidity, 13 hours of leaf wetness and 20 to 28°C to germinate (Lipps *et al.*, 1998; Ward *et al.*, 1993). Lesions of GLS develop on leaves and produce conidia for secondary spread after two to four weeks (Lipps *et al.*, 1998). Caldwell *et al.* (2002) reported that disease severity increased with increasing levels of soil nutrients.

The tan to brown, and narrow lesions with squared-off ends are visible after tasselling (Wysong, 1996; Ward *et al.*, 1993). These lesions grow together resulting in larger blighted areas and the dense sporulation produces a greyish cast on leaves, ear husk and stalks leading to stem lodging (Ward and Birch, 1993). Early blighting of the leaves above the ear causes grain yield loss but late infection does not cause economic damage (Lipps *et al.*, 1998). Ward *et al.* (1993) estimated grain yield loss at 50% in the humid and high potential areas. A viable management option would be to delay onset of the disease for as long as possible, through conventional tillage that reduces the level of soil based inoculum (Carson, 1999; Lipps *et al.*, 1998; Ward *et al.*, 1997d). Resourceful farmers can apply foliar fungicide sprays to control GLS (Ward *et al.*, 1996; Ward *et al.*, 1997a, 1997b, 1997c; Lipps *et al.*, 1998; Ward and Newell, 1998; Carson, 1999), but these fungicides are usually not affordable by small-scale farmers in Southern Africa. Thus, planting of resistant cultivars would provide a more sustainable control strategy.

1.7.2 Sources of Resistance to Gray Leaf Spot

Resistant germplasm has been found in the United States and South Africa. In the USA, Ayers *et al.* (1984) and Freppon *et al.* (1994) reported fleck-type lesions in the resistant inbreds Pa875, NC264, NC288, Va59 and Oh43. Freppon *et al.* (1994) also reported some chlorotic lesions on resistant inbreds NC250 and NC288 and some moderately resistant hybrids. These chlorotic lesions provide a mechanism for reducing the number and size of lesions and reduce secondary inoculation. Resistance was also reported in the inbreds T222 and Mo18W (Ulrich *et al.*, 1990), and some inbreds derived from Lancaster population (Graham *et al.*, 1993). Graham *et al.* (1993) reported that inbreds derived from Lancaster had better resistance than inbreds from Iowa Stiff Stalk Synthetic (BSSS) population. In South Africa, Gevers *et al.* (1994) reported GLS resistance in the inbreds K054W and S0507 from the F and M heterotic groups (Section 1.2 in Table 1.1). Gevers *et al.* (1994) reported that inbred S0713W from the P heterotic group was susceptible to GLS. Thus, GLS resistance is also available in locally adapted maize germplasm.

1.7.3 Gene Action Conditioning Gray Leaf Spot Resistance

Gene action conditioning resistance to GLS has been studied extensively in temperate materials. Bubeck *et al*. (1993) reported that more than five genes controlled resistance in the line NC250A indicating that resistance was quantitative. Larger general combining ability (GCA) than specific combining ability (SCA) effects for GLS resistance have been reported (Huff *et al*., 1988; Thompson *et al*., 1987; Ulrich *et al*., 1990). Predominance of the GCA variance over the SCA variance suggested that additive effects were more important than non-additive gene action in conferring resistance to GLS. However, a study by Elwinger *et al*. (1990) and Freppon *et al*. (1994) found that hybrids between resistant and susceptible inbreds were resistant suggesting that dominance gene action was also important in conditioning resistance to GLS. Coates and White (1998) also reported that dominance gene action controlled GLS resistance in the inbred B37*HtN.* Studies by Lipps *et al*. (1998) indicated that environmental effects were not significant for GLS resistance because hybrid rankings were similar across 22 environments. In Southern Africa, Gevers and Lake (1994) reported larger GCA than SCA effects, but Hohls *et al*. (1995) reported complete dominance and minor epistasis in inbreds from the P, M and F heterotic groups. Resistance to GLS could be improved through reciprocal recurrent selection that utilises both GCA and SCA variation.

1.7.4 Selection for Resistance to Gray Leaf Spot

Selection for resistance can be effective because additive genetic effects largely determine GLS resistance and resistance is highly heritable (Donahue *et al*., 1991). Coates and White (1998) reported narrow-sense heritabilty of 100% in some inbreds of B73 orientation. Cromley *et al*. (2002) reported high level of resistance in single crosses between resistant and susceptible inbreds, which was similar to crosses between two resistant parents. Graham *et al*. (1993) reported that 11 cycles of recurrent selection for grain yield did not compromise GLS resistance in the population BSSS. However, Lipps *et al*. (1998) reported that some resistant hybrids had lower yield potential than their susceptible hybrids. According to Lipps *et al*. (1998), resistant hybrids only had yield advantage under high GLS severity. Appropriate resistance for use in regional maize would be one that does not result in a yield penalty when the disease is not severe. This is very important because in

Southern Africa GLS severity varies between seasons depending on the highly variable weather pattern.

1.8   Phaeosphaeria Leaf Spot Disease

Phaeosphaeria leaf spot (PLS) is caused by the fungus *Phaeosphaeria maydis* (Henn.) Rane, Payak and Renfro, and is listed among diseases of minor importance in the USA (Carson, 1999). The PLS causes small and pale green or chlorotic lesions on leaves. These lesions start as some scattered spots on the leaves and then coalesce. In sub-Saharan, Africa PLS has been widely reported in Cameroon, Kenya, South Africa and Zimbabwe (Carson, 1999). Despite its widespread occurrence, PLS appears not to cause significant yield reductions except in Brazil. Carson (2005b) reported that PLS occurred in late grain filling resulting in 11 to 13% yield reduction on the most susceptible hybrids. In Brazil, Paccola-Meirelles (2001) reported yield reduction of 63% in susceptible cultivars. In addition, Pegoraro *et al*. (2001) reported a significant correlation (r = 0.45) between grain yield reduction and PLS severity in Brazil. This indicated that PLS has the potential to reduce grain yield. Although grain yield losses have not been quantified, recent observations have shown that the incidence of PLS is on the increase in east and Southern Africa.

Resistance of maize cultivars to PLS has been evaluated in the USA, Brazil and South Africa. The inbred Mo17 and its derivatives were more resistant than B73 and its derivatives (Carson, 1991, 2001). Even though Carson (1999) reported that some widely used lines were susceptible, only a few hybrids were severely affected by PLS. In South Africa, Flett (2004) reported the highest disease rating of 9.35% on maize hybrids and he concluded that most hybrids were resistant.

Inheritance of PLS resistance has been studied in the USA and Brazil. In the USA, Carson (2001) concluded that additive variation and small dominant gene action, which involved three to four genes controlled resistance to PLS. In Brazil, Pegoraro *et al*. (2002) reported that additive gene action involving at least two major genes conditioned resistance. Carson (2001) also reported that heritability was high (0.70 to 0.85). High heritability and predominance of additive variation suggested that resistance could be improved by selection. Despite the increasing incidence of PLS, there are limited studies that have been conducted on the diverse germplasm in sub-Saharan Africa. Thus, in the current study germplasm drawn from major heterotic groups in Southern Africa was evaluated for resistance.

1.9   The Drought Problem

An agricultural drought is defined as lack of adequate soil moisture for a given crop to grow and thrive during a particular time. Apart from reducing agricultural productivity leading to food security problems, drought has some ripple effects on the agriculture-dependent sub-Saharan economies (Richardson, 2003). Banziger and Diallo (2002) reported that 93% of maize production was on dry land. Thus, there is very limited use of irrigation in the region where drought is rampant. In Southern Africa, the most devastating drought was recorded in 1991/1992 season and reduced grain production about 60% (Rosen and Scott, 1992).

1.9.1   Managing Drought

On the farm level, sustainable strategy for mitigating yield losses due to drought should be based on use of tolerant cultivars. According to Boyer (1992), breeding for high water use efficiency improves economic yield. Improvements for drought stress tolerance results in cultivars with better yield and growth under drought condtions. Boyer (1992) classified mechanisms of drought tolerance in cultivars as dehydration avoidance and dehydration tolerance under drought conditions. Structural mechanisms such as improved rooting depth and increased cuticle thickness delay dehydration, but grain yield is reduced due to increased partitioning of dry mass towards production of structures. According to Pingali and Pandey (2001), farmers in drought prone areas can by plant early such that their cultivars would flower during high moisture conditions and thus escape the drought. Use of conventional tillage increases water infiltration into the soil, encourages development of deep roots. Breeding for early maturing cultivars would be feasible the flowering traits such as anthesis to silking interval and days to flowering are highly heritable, even under drought stress conditions (Pingali and Pandey, 2001). The problem reported by Pingali and Pandey (2001) that these early maturing cultivars incurred a yield penalty when grown under favourable rainfall conditions. Thus, it is important to study physiological mechanisms that condition cultivar resistance to low moisture stress.

1.9.2   Physiological Basis of Yield Reduction

Scientists are in agreement that early reproductive development is most vulnerable to water deficits, because grain yield reduction is not reversible if drought stress

coincides with flowering (Boyer, 1992; Bolanos and Edmeades, 1993a). Thus highest yield reduction occurs at flowering stage because of abnormal floral, ear and kernel development (Westgate and Boyer, 1986; Lafitte and Edmeades, 1995; Edmeades *et al*., 1999; Zinselmeier *et al.,* 2002). Low moisture stress at flowering reduces the rate of photosynthesis to almost zero under mild and severe stress (Schussler and Westgate, 1994; Westgate and Boyer, 1986; Zinselmeier *et al*., 1999; Zinselmeier *et al*., 2002).

Low moisture stress during the reproductive stage reduces sink strength and kernel development. Setter *et al*. (2001) reported that moisture stress at pre-pollination reduced accumulation of carbohydrates in apical and basal florets. Vasal *et al*. (1997) reported that assimilates were preferentially distributed to the tassel resulting in poor seed set. Setter *et al*. (2001) reported that water deficit increased abscisic acid (ABA) concentration in the reproductive tissues. According to Boyer (1992), high ABA level inhibited endosperm cell division and reduced seed set. Thus ABA, probably plays a critical role in controlling drought tolerance. Zinselmeier *et al*. (1999) reported that water deficit resulted in abortion and few kernels. Previously, Zinselmeier *et al*. (1995) had reported that moisture stress inhibited ovary growth, decreased levels of reducing sugars, depleted starch and inhibited the activities of acid invertase, which maintains the reproductive sink strength and facilitates early kernel development.

1.9.3 Sources and Gene Action Conditioning Drought Tolerance

Generally, the best sources of drought stress tolerance should have exceptionally high agronomic performance and large genetic variance for other important traits. According to Vasal *et al*. (1997), inbred parents should be preferred, because heritability increases with inbreeding levels. Vasal *et al*. (1997) reported that inbreeding of segregating populations resulted in high frequency of inbred lines with long anthesis to silking interval (ASI) under drought. Secondary traits such as the ASI are correlated with grain yield under drought stress, and are easy to measure (Bolanos and Edmeades, 1996; Banziger *et al*., 2000). In another study, Betran *et al*. (1996) reported that there was a low correlation (0.40) between the grain yield of inbred parents and grain yield of their testcrosses under drought conditions. Thus, the crosses between drought tolerant inbred lines still have to be tested for tolerance to drought.

A survey of literature showed that there is little research on gene action conditioning drought stress tolerance in Southern African maize germplasm. Betran *et al*. (2003a) reported predominance of additive gene action in controlling grain yield in tropical germplasm under drought stress. However, in another study, Betran *et al*. (2003b) reported significant non-additive effects for grain yield under drought conditions. These results were consistent with Guei and Wassom (1992) who also reported predominance of additive gene action in controlling flowering traits, while dominance was more important for grain yield and number of ears per plant under drought stress. Studies of quantitative trait loci also confirmed the importance of both additive and dominance action in conditioning yield and the associated flowering traits (Agrama and Moussa, 1996). This suggests that breeders should utilise selection strategies such as reciprocal recurrent selection and hybridisation that employ both additive and dominance gene action in improving maize for drought tolerance.

1.9.4 Selection for Drought Stress Tolerance

Progress in improving maize for drought stress tolerance, especially in Southern Africa has been slow. This has been partly attributed to the large G x E interaction in the highly variable production environments of small-scale farmers. Rainfall amount and timing are highly variable such that it is difficult to predict the occurrence and the severity of drought stress in these environments (Pingali and Pandey, 2001). In addition breeding is made complicated by the low heritability for grain yield under the drought conditions (Bolanos *et al.*, 1993; Byrne *et al*., 1995). However, research has indicated that grain yield can be improved under drought improved by selecting for the highly heritable secondary traits like the ASI and number of ears per plant. These traits have been confirned to be highly correlated with grainyield under drought stress conditions (Bolanos and Edmeades, 1993a; Chapman and Edmeades, 1999; Pingali and Pandey, 2001; Tollenaar *et al*.; 1992).

Selection for yield under managed stress results in better breeding progress than selecting under non-stress conditions. Edmeades *et al*. (1999) reported yield gains of 3.8 to 12.6% per cycle in tropical populations following simultaneous selection under well-watered and managed drought stress at flowering. Byrne *et al*. (1995) reported higher progress under managed drought stress (1.68%) than in multi-location trials (1.06%) in Tuxpeno Sequia and Tuxpeno confirming previous reports by Bolanos and Edmeades (1993b). However, Byrne *et al*. (1995) reported that selection under water stress only might result in cultivars with lower yield under favourable conditions.

Boyer (1992) suggested multilocation testing at varying water regimes to avoid selecting cultivars that incur a yield penalty under favourable conditions. According to Boyer (1992), multilocation testing can identify cultivars with the following combinations: a) "high yielding in both optimum and water-deficit; b) high yielding in optimum, but low yielding under water deficit; and c) low yielding in optimum, but high yielding under water deficit conditions." As a result Boyer (1992) suggested that selections should be made under drought conditions followed by testing in multilocation trials. Byrne et al. (1995) also suggested a similar two step approach with the first aimed at reducing ASI by evaluating lines at a few sites under managed drought stress at flowering and then select for specific adaptation and high yield potential in multilocation trials. An integrated approach that begins with screening under managed drought stress before multilocation testing is thus suggested.

1.10 Summary

Generally, this review showed that there is very little published information for research conducted in sub-Saharan Africa. Genetic information obtained from outside the sub-continent might not have direct application, because the agricultural systems and environments are quite different.

A huge gap still exists between grain yield potential and the actual yield, indicating the existence of opportunities for grain yield improvement. Improvement in yielding ability of temperate maize was associated with increasing stress tolerance, especially under high plant density. Thus, it can be suggested that breeding for baseline tolerance to drought and disease stress would contribute towards reducing the yield gap in sub-Saharan African environments. In terms of gene action, the additive variance, dominance and epistasis effects played significant roles in conditioning yield and its associated traits. This in turn suggests that simple selection alone, which exploits additive effects, would not be adequate to improve yield. Breeding for stress tolerance and wide testing with few replications could identify cultivars displaying high yield stability and high yield potential. Low negative correlation was reported between yield potential and yield stability as measured by regression, suggesting that high yield potential and high yield stability may not be mutually exclusive.

Genetic variation for resistance to GLS was shown in both temperate and Southern African adapted germplasm. Resistance to GLS was highly heritable and

predominantly controlled by additive effects. Dominance action displayed by some populations indicates that both inbreds and crosses should be evaluated for resistance. It was shown that there was no direct relationship between GLS resistance and grain yield when the disease pressure was low. However, a negative relationship would be expected when the epidemic is moderate to severe, especially if it occurs before the grain filling stage.

Although there was limited literature on PLS, the few publications reported high levels of resistance to PLS in some USA and South African materials. Resistance was inherited mainly in an additive manner and was highly heritable; suggesting that resistance in regional maize can be enhanced by selection. Significant grain yield reductions that were correlated with PLS severity were found in Brazil, indicating that the disease has the potential of posing threat to regional food security. Information on the epidemiology of this disease was also limited.

Grain yield loss was largest when drought occurred at flowering stage due to abnormal floral, ear and kernel development. Grain yield has a significant correlation with ASI and number of ears per plant under drought stress. This suggests that yield can be improved by selecting for short ASI and high number of ears per plant under drought stress. Although few studies were reported, additive effects under drought stress predominantly influenced inheritance of grain yield. It is suggested that incorporating tolerance in parent-inbred lines would enhance performance of hybrids. This review also suggested that cultivars with high yield potential under both optimum and drought conditions would be obtainable by selecting first under drought stress and followed by selection in multilocation trials that represent the target environments.

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Chapter 2: Farmer Perceptions on Maize Cultivars in the Marginal Eastern-Belt of Zimbabwe and their Implications for Breeding

Abstract

Productivity of maize is low in the smallholder sector because the crop is grown under stress-prone environments and limited resources. The objective of this study was to investigate farmer perceptions on maize cultivars, constraints and preferences, and their implications for breeding. Participatory rural appraisal (PRA) and household formal surveys were conducted in the marginal eastern-belt of Zimbabwe, during 2004 to 2005. Sample districts for the study were Mutasa, Mutare West, Chimanimani and Chipinge. The PRA tools that were employed consisted of problem listing, analysis and ranking by farmers and key informants. Semi-structured questionnaires were designed to guide the discussions and farmers were provided with sufficient opportunity to bring up their own issues. Although results revealed that farmers predominantly grew hybrids, production and grain yield were low (240 to 500 kg/ha) and below the national average. Due to the different agro-ecologies, production constraints differed between survey districts influencing farmers' preferences for cultivars. In the relatively more productive Mutasa, farmers preferred weevil resistant cultivars, whilst those in the severely drought-prone Chipinge and Mutare West preferred cultivars with drought tolerance among other traits. Farmers had strong preference for the old hybrids of the 1970's to new hybrids of the 2000's, due to their perceived superior drought tolerance. This suggested that progress has not been made over the past 35 years in breeding cultivars with drought tolerance for deployment in these marginal areas. Farmers also preferred their local landrace "*Chitonga*", because of its superior taste and flint grain, but they recognised that it had a long maturity period, thus, failed to fit into the short growing seasons. Improvement of this local cultivar would enhance households' food security and the livelihoods of resource poor farmers in Chipinge and Chimanimani, with possible spill over effects into the neighbouring Mozambique where this cultivar is also grown. This study revealed that farmers preferred cultivars that combine high yield potential, early maturity and drought tolerance in all areas; and tolerance to low soil fertility and weevil resistance in the relatively wet areas with potential for surplus production. It is thus implied that breeders should aim at developing cultivars combining these traits.

Keywords: Maize, PRA, Preference, Production Constraints, Stress Tolerance

2.1   Introduction

Small-scale farmers in marginal areas dominate the production of maize, a staple food crop in Southern Africa. Grain yield of maize is low due to the variable production environment, stress and limited access to essential inputs (Banziger and de Meyer, 2002). The maize yields are low and averaging below 1.2 t/ha (FAOSTAT, 2003) notwithstanding the more than 60-year history in crop research and development in the region. It is believed that some superior cultivars have not been adopted because of lack of sufficient consideration of farmers' preferences in their breeding process. In most cases, commercial breeders fail to consider the special and unique preferences of small-scale farmers in marginal areas possibly because they are not aware of them (Toomey, 1999; Banziger and Cooper, 2001). Thus, effective breeding should be firmly based on clear identification of farmers' perceived constraints and their preferences for cultivars through interactive breeding and research. In participatory crop breeding, farmers can provide important information on plant types, desired traits and insight into trade-offs they are willing to make in designing cultivar for their area (Sperling *et al*., 2001).

Small-scale farmers' involvement in participatory crop breeding is not new. For instance, Banziger and de Meyer (2002) reported that farmers participated in the evaluation of pre-selected maize cultivars in CIMMYT's (International Maize and Wheat Improvement Center) mother-and-baby trials in Southern Africa. Previously, farmers were reported to have been involved in rice varietal selection in India and Nepal (Joshi and Witcombe, 1996; Sthapit *et al*., 1996; Witcombe *et al*., 1996). More recently, Monyo *et al*. (2001) reported that farmers were engaged in pearl-millet (*Pennisetum glaucum* L.) cultivar selection in Namibia.

What might be most appealing to the small-scale farmers in Southern Africa are Sedgley's (1991) market and stress ideotype concepts and not Donald's (1968) wheat- and Mock and Pearce's (1975) maize-ideotype concepts, which describe optimum plant types - the preferred option for plant breeders. The optimum plant type of Donald (1968) and Mock and Pearce (1975) describes a plant design that maximises photosynthetic efficiency due to their upright leaves and a large sink resulting in high harvest index. However, their optimum plant requires optimum fertiliser, pesticides, weeding and water, which makes it an option only for the resourced or large-scale commercial farmers. The small-scale farmers in marginal areas have limited access to these resources; hence they would not exploit the

benefits of the optimum plant type. These farmers would require a market and stress ideotype. According to Sedgley (1991), the market ideotype identifies desirable traits such as quality, while the stress ideotype identifies the characters required to fit the cultivar into its target environment, especially climate, soil, disease and pests. For example, de Groote *et al*. (2000) reported that farmers in eastern Kenya preferred early maturing cultivars ahead of high yield potential. Similarly, Banziger and de Meyer (2002) reported that farmers in Southern Africa preferred early maturing cultivars with hard endosperm and good husk cover. These results do not have a global application, but would only pertain to specific areas due to different environmental and socio-economic conditions. The objective of the current study was to investigate farmers' perceptions for maize cultivars in the marginal eastern-belt of Zimbabwe and their implications for breeding.

2.2   Materials and Methods

2.2.1 Study Area

The study was conducted in the Chimanimani, Chipinge, Mutasa and Mutare West (Marange) districts, of the Manicaland Province[1] of Zimbabwe, during 2004 to 2005. Population and number of households in the sample districts are shown in Table 2.1. The area falls within the rain shadow of the humid and misty eastern highlands. Rainfall amount and pattern is modified by altitude, such that high elevation areas receive more rain than lowland areas. Agro-ecological regions are thus demarcated into five regions according to relief, rainfall adequacy and efficiency. Thus, Natural Region I receives the highest and most reliable rainfall, while Natural Region V has the least reliable and erratic rainfall amount and its distribution over the season (Vincent and Thomas, 1961). The area covered by the current study comprised Natural Regions IIb to V; stretching for ±200 km from Mutasa (North of Mutare City) to Chipinge District in the south and abreast covering Save and Odzi River catchments. Altitude falls significantly from above 800 m in Mutasa down to about 430 m at Middle Save in Chipinge. The study area represented agro-ecological zones within the mid-altitude dry and lowland tropical dry macro-environments (Appendix 1). In this area, rainfall is very erratic and crop production is to a great extent dominated by small-scale or resource poor farmers.

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[1] The Manicaland Province is to the east of Harare and west of Mozambique.

Table 2.1: Household data for sample districts (Central Statistical Office, 2004)

District	Males	Females	Total	Number of Households
Mutare Rural	106061	116322	222383	48631
Chipinge	134904	148888	283792	61860
Chimanimani	55494	59803	115297	26524
Mutasa	78470	88176	166646	39629

2.2.2 Sampling Procedures

In order to capture the expected variability in agro-ecological and socio-economic environments, the four districts situated to the North (Mutasa), South (Chipinge and Chimanimani) and West (Mutare West) of the Provincial Capital of Mutare were selected by stratified sampling. The eastern side of the City could not be sampled because it is in Mozambique. The northeast and southeast parts are in the very high potential Natural Region I under high value large-scale commercial and plantation agriculture.   Since the districts cut across all the agro-ecological regions in the country, further stratified sampling was applied in the selection of villages within each district to capture those within Natural Regions IIb, III, IV and V where maize is grown under moderate to severe moisture stress conditions (Appendix 1). In each of the villages, at least six farmers were randomly selected from lists provided by the local extension staff (Appendix 1). Additionally, three focus group discussions were held in the Save River Valley area.

2.2.3 Data Collection

Primary data were collected through both formal household survey and the informal Participatory Rural Appraisal (PRA) tools. Local extension staff, councillors and village headmen facilitated the survey by creating a good rapport with local people, mobilised farmers for the focus group discussions and provided lists of farmers to be sampled for the formal survey. The PRA involved three focal group discussions and interviews with key informants such as local teachers, businessmen, school headmasters, councillors and agricultural extension staff in the Save Valley area of Chipinge and Chimanimani. The technique employed consisted of problem listing, analyses and ranking by key informants using semi-structured questionnaires. These were designed to guide the discussions yet provided the group sufficient opportunity to bring up their own issues. In general, discussions started by asking farmers to list uses of maize and identify competing cereals and leguminous crops they grew in

their area. Secondly, farmers were asked to list and rank key constraints to maize production. Thirdly, farmers listed cultivars they had grown, ranked them and identified preferred traits of stress tolerant cultivars. Farmers were also asked to list and give reasons for cultivars they would like to grow again and those they would never grow again. In addition, seed issues were discussed at great depth. Throughout discussions a local extension staff member guided the process, while enumerators concentrated on taking notes. Issues that were raised during focus group discussions were taken up for further analyses with local opinion leaders.

Prior to the formal survey, a pilot study was conducted involving 53 households in Marange, Honde Valley and Birchenough Bridge in Mutare West, Mutasa and Chipinge, respectively (Appendix 1). As indicated in Appendix 1, a total of 93 households in Mutasa, Chipinge and Mutare West participated in the formal survey designed to dissect issues raised during the PRA. The field research team comprised a principal investigator, three enumerators and a local extension staff. The medium for discussion was the local dialect "Shona" (i.e. *ChiManyika* and *ChiNdau*, in the North and South, respectively), which is widely spoken in the area. Incidentally, all the members of the research team spoke Shona and thus eliminating the need for translation and its associated errors. To eliminate gender dominance in discussions expected at Nyakumanwa Village and Changazi Ward 20, in Chipinge and Chimanimani, respectively, separate discussions were held with men and women farmers. This was necessary because in the Ndau culture men are dominant over women such that women would not speak freely in the presence of men.

Both formal and informal approaches were employed in data collection in order to enhance precision and high evidential value. According to Mergeai *et al*. (2001), informal PRA approach enhances evidential value by taking care of relevant situational local knowledge and identifying key elements, while greater precision is obtainable from formal surveys. Data generated were compared and the final synthesis of the information is presented.

2.2.4 Data Analyses

Statistical analyses of both quantitative and qualitative data were performed in SPSS (Release 11.5) computer package (SPSS Inc., 2002). Descriptive statistics, analysis of variance and mean comparisons were computed for data collected in each district followed by mean comparisons between districts.

2.3 Results

2.3.1 Features of Farm Economy

The PRA study established that maize was a significant staple food in the area. The uses of maize ranged from *Sadza* (the staple meal) through traditional beer brewing to snacks (both as fresh and dry grain). The household and farm economy data are presented in Appendix 2. Household sizes were similar in Chipinge and Mutasa, but were smaller than in Mutare West. The average land holding differed significantly between districts, ranging from 1.3 in Mutasa to 2.2 ha in Chipinge (Appendix 2). Whereas in Chipinge and Mutasa the dominant crop was maize, in Mutare West it was sorghum (*Sorghum bicolour* L.). All leguminous crops were grown as minor crops throughout the districts. Sorghum was perceived to be the best crop for the area at Nyakumanwa Village, but pearl-millet was ranked as the best crop at Changazi (Table 2.2). Groups of farmers agreed that maize was not as drought tolerant as sorghum and pearl-millet, but key informants argued that maize has generally been accepted in the area. In spite of its lack of tolerance to drought stress, farmers showed great interest in the maize crop.

Table 2.2: Rank of crops for preference by farmers in Chipinge and Chimanimani

Crop	Changazi	Nyakumanwa
Sorghum	2	1
Maize	3	2
Sunflower	4	3
Pearl-millet	1	4
Finger millet	5	5

Scores: 1 = bestt and 5 = least preferred crop for the area

2.3.2 Maize Production

Maize grain production differed significantly (P<0.05) between locations during 2002 to 2004 period with Mutasa having highest yields (Table 2.3). Only farmers in Mutasa district had grain surplus of about 100 kg each in 2004 (Table 2.3). Productivity data confirmed that for the past three years drought was most severe in 2004. Furthermore, the majority (62%) of farmers regarded 2004 as the worst drought-year in three years, while 21% expressed the view that 1992 was the worst drought-year in the area.

Table 2.3: Grain production, productivity and consumption per household

	Mutasa	Chipinge	Mutare West	Overall	F. Probability
Grain Production (kg)					
2004	595	255	55	285	0.00
2003	670	380	55	345	0.00
2002	765	505	240	480	0.00
Grain sold (kg)					
2004	105	0	0	30	0.00
2003	160	15	0	55	0.00
2002	240	30	10	85	0.00
Grain Consumption/Month (kg)					
Average	42	26	42	36	0.08
Grain Yield (kg/ha)					
2004	578	46	103	243	
2003	649	237	101	329	
2002	745	317	439	500	

2.3.3 Production Constraints

Results showed significant differences in ranking production constraints between districts (Table 2.4). Overall results indicated that non-availability of seed on the formal market followed by drought were the most important constraints. Farmers reported that commercial seed was not available on the formal market during 2002 to 2004 seasons. However, farmers in Mutare-West ranked drought first, followed by non-availability of seed as major constraints in the area. In Chipinge and Mutasa, farmers identified drought and low soil fertility as most important, respectively (Table 2.4). Household data and the focus group discussions in Chipinge (Nyakumanwa, Kondo and Changazi Villages) identified drought as more important than low soil fertility (Table 2.5). Farmers, especially in Chipinge perceived that their soils were of good fertility (Appendix 3). Opinion leaders confirmed farmers' perception that their soils were highly fertile to the extent that they sold the free packs of fertiliser from donors. Farmers also perceived that use of inorganic fertiliser would damage their soils. Although farmers believed that their soil was fertile, the household survey revealed that they applied ±32 wheel barrows (±1500 kg) of cattle manure and 125 to 250 kg of inorganic fertiliser per hectare as basal and top dressing.   The survey data also showed that the rainy season started in November and ended in March during 2002 to 2004 seasons (Appendix 3). Farmers in Mutasa rated rainfall amount as moderate, but in Chipinge it was reported that rainfall amount was too little for the maize crop. In general, drought occurred during mid to late season with different intensity. It was rated as moderate in Mutasa and severe in Chipinge and Mutare West (Appendix 3).

Table 2.4: Mean rank for perceived production constraints in formal survey

Constraint♠	Mutasa	Chipinge	Mutare West	Overall	F. prob.
Seed Availability	1.5	1.3	2.3	1.7	0.00
Drought	3.0	2.3	2.0	2.4	0.00
Poor Soil Fertility	2.2	3.2	2.8	2.8	0.00
Cultivar Problems	4.5	4.4	4.5	4.5	0.97
Disease and Insect Pests	4.1	5.1	4.3	4.5	0.02

♠Characteristic with smallest mean within a column was perceived to be most important

Table 2.5: Mean rank for perceived production constraints in focus group discussions

Constraint♠	Nyakumanwa	Kondo	Changazi	Key Informants
Low rainfall	1	1	1	1
Non-availability of seed	-	2	2	2
Salt in isolated areas	-	-	4	-
High heat stress	-	-	3	3
Draught power	-	3	-	-
Low soil fertility	5	4	5	5
Land too small	4	5	-	-

♠Scores used were: 1 = most important and 5 = least important; - = No information available.

2.3.4 Maize Cultivars Grown

Results from the household survey indicated that farmers predominantly grew hybrids of the following brands: Seed Co (SC), Pannar (PAN) and Pioneer (Appendix 4). Additional cultivars that were mentioned included R201, R200, R215 and the dwarf hybrids. In Chipinge and Chimanimani (Changazi Ward 20), farmers also mentioned a traditional land-race called "Chitonga", but they recognised that it was not the ideal cultivar.   There was a common perception that "Chitonga" required higher rainfall, since it was late maturing than hybrids SC403 and PAN 413 that were grown in the area. Another attribute that was disliked by farmers was that it was too tall compared to the hybrids. The preferred attributes of "Chitonga" were better taste, flintier grain and better resistance to grain weevils than commercial hybrids grown in the area. Farmers reported that "Chitonga" was also a common cultivar in Mozambique. Thus, farmers obtained the seed from Mozambique through Ndowoyo area in Chipinge and Rusitu Valley in Chimanimani.

Farmers and the opinion leaders showed very high regard for old hybrids of the 1970's. Although they have accepted the new early maturing hybrids, the general perception was that these were not as drought tolerant as the old hybrids. However,

opinion leaders mentioned that even the old hybrids could still be improved by imparting the "sorghum-type" of tolerance. "Sorghum-type" of tolerance was defined as "the ability of a drought stricken cultivar to recover when the rain resumed after the mid-season drought. Their explanation was that the rainy season begins very well in November, with good precipitation continuing into December and a drought spell occurs in January. When rainfall resumes in February, sorghum has the ability to recover, but not maize. This implied that January is the "black" month in the area, and an "ideal" cultivar would combine early maturity and drought tolerance at flowering. In addition, opinion leaders mentioned that the ideal cultivar should also have heat stress tolerance due to very high temperatures during summer.

2.3.5 Ranking of Cultivar Traits and Preferences

Except for maturity period and yield, farmers showed significant differences in ranking of cultivar trait preferences between districts (Table 2.6 and 2.7). Although the whole sample level analysis showed high yield as the most important criterion used in varietal selection and drought as third, farmers in Mutare West identified maturity period as the most critical factor. In Mutasa, farmers ranked grain weevil and disease resistance as third ahead of both tolerance to drought and low soil fertility stress. In the absence of a truly drought tolerant cultivar, farmers preferred to plant sorghum than maize (Table 2.3). In general, farmers in all districts were not concerned much about prolificacy (number of cobs per plant), cob size, husk cover and pounding ability (Table 2.6). There were no significant differences ($P>0.05$) between districts for grain texture preferences (Table 2.6). Thus, consistently, farmers preferred semi-dent and flintier grain to the dent. At Changazi, farmers were of the opinion that semi-flint grain texture conferred high storability, better taste and high yield due to its superior test density (weight per unit volume). Generally, farmers preferred long thin cobs compared to long and fat.

Table 2.6: Mean rank values for preferred traits of stress tolerant cultivars from formal survey

Characteristic[♣]	District			Overall	Probability
	Mutasa	Chipinge	Mutare		
General Traits					
High yield	2.7	1.8	2.8	2.4	0.07
Maturity Period	3.2	2.5	2.6	2.8	0.35
Drought stress tolerance	5.3	3.1	3.8	4.1	0.01
Low soil fertility tolerance	5.0	5.6	4.0	4.9	0.00
Grain weevil resistance	3.6	4.3	6.7	4.9	0.00
Cob size	7.0	6.3	3.6	5.5	0.00
Disease resistance	3.8	4.6	7.9	5.6	0.00
Number of cobs per plant	8.4	7.1	6.0	7.1	0.00
Cob husk cover	6.5	7.7	8.3	7.5	0.00
Pounding ability	9.6	10.0	8.8	9.4	0.02
Ear or Cob Aspect					
Long thin	1.6	2.2	1.5	1.7	0.00
Medium	2.1	2.1	2.1	2.1	0.99
Long fat	2.3	1.6	2.5	2.2	0.00
Grain Texture					
Flint	1.8	1.6	1.8	1.7	0.49
Intermediate	1.7	1.8	1.7	1.7	0.98
Dent	2.5	2.6	2.5	2.5	0.71

[♣]Characteristic with smallest mean rank is the most important in each column.

Table 7: Mean rank values for preferred traits of cultivars from focus group discussion

Trait[♣]	Nyakumanwa	Kondo	Changazi	Key Informants
High yield	3	1	3	3
Drought tolerance	1	2	1	1
Early maturity	2	3	2	2
Resistance to insects	4	4	-	4
Disease resistance	5	5	-	5
Cob size	-	5	-	5
Low soil fertility tolerance	5	5	-	5

[♣]Scores used were: 1 = most important, 5 =least important; - = No information available

2.4   Discussion

2.4.1 Production and Constraints

Results indicated that Mutasa was the wealthiest district with respect to the high number of cattle, television sets and modern houses in the area. There was at least one radio set per household in all districts suggesting that extension communication

can be effectively transmitted via the radio. It is difficult to explain the low average land area that is planted to pearl-millet in Mutasa. In Chipinge, the traditional authorities, especially in Chief Musikavanhu's area of jurisdiction prohibited pearl-millet cultivation. According to key informants, pearl-millet was regarded as taboo in the area, because the chief does not eat pearl-millet food or beer brewed from it.

Farmers in Mutasa reported relatively higher grain yield than the other areas under study. These farmers sold at least a 100 kg each of grain indicating that the area has the potential to produce surplus grain despite the late season drought. Differences in productivity among the districts could also be explained by the different rainfall patterns, nature and intensity of drought in the area. Mutasa experienced moderate late season drought, while the other two districts reported severe drought at anthesis, which is a very critical stage in grain yield formation. Flowering is the most critical stage associated with greatest yield loss, especially when drought occurs during the reproductive stage (Cakir, 2004; Campos *et al.*, 2004). This has serious implications for food security in the area, given that average household grain consumption was estimated at 40 kg per month. An average household with eight people (Appendix 2) required at least 480 kg of grain per annum, which is above the average yield or total production in Chipinge and Mutare West in 2004. This suggests that there was serious grain deficit in Chipinge and Mutare West in 2004.

In Mutasa, farmers mentioned low soil fertility as a major production constraint, which together with late season drought, could partly explain the low yields obtained in the area (578 to 745 kg/ha in Table 2.3) compared to the national average for Zimbabwe (±1000 kg/ha). This district was relatively more productive than Chipinge and Mutare West hence these farmers had a different perception on drought and low soil fertility. Although they experience late season drought, they receive relatively higher rainfall early in the season that might cause some leaching of soil N, whereas less leaching is expected in the drier districts of Chipinge and Mutare West. Perhaps the natural floods that have occurred over the years, such as in 2000, deposited large and fertile silts in the lowland areas like Save Valley in Chipinge.

2.4.2 Preferred Traits and Maize Cultivars

Farmers' strong preference for old hybrids of the 1970's over the current new hybrids suggested that little progress has been made in breeding drought tolerant cultivars for deployment in these marginal areas. Breeders appear to have focussed on

breeding for high potential areas and disease resistance. Thus, there is need to refocus the breeding goals in order to find stress tolerant cultivars for production in marginal areas. Farmers' high preference for their land-race "Chitonga", suggested that scientists would make impact by improving or breeding from this cultivar, because it is already accepted in the area. Alternatively, breeders can incorporate the desired traits in other elite cultivars. Improvements would be aimed at reducing plant height and maturity period, so that it can fit into the ultra short seasons. The attributes to be maintained would be its flint grain texture and "good" taste. In addition this cultivar has to be evaluated for agronomic performance and study the genetic effects controlling yield and other traits under drought, which is prevalent in the area.

Although farmers indicated that they grew at least 10 kg of hybrid seeds (data not shown), it was not established whether they grew first (F_1), second (F_2) or later generations of the hybrids. The study could only speculate that few farmers were planting F_2 hybrid grain as seed, because sixteen percent of farmers in Mutasa indicated that they grew farm saved seed. Unfortunately, hybrid vigour or heterosis that confers high yield in F_1 hybrids declines sharply by over 50% in F_2 and subsequent generations (Falconer, 1981). If farmers were growing F_2 or later generations of hybrids, then this could partly explain the below national average yield in Mutasa and perhaps in the other districts too.

Non-preference of drought stress tolerant cultivars in Mutasa could be explained by the moderate drought stress, compared to severe stress in Chipinge and Mutare West. Given the differences in rainfall pattern, the results suggested that an early maturing cultivar would most likely escape late season drought in Mutasa, but would be affected by drought at flowering in Chipinge and Mutare West. Possibly because of excess production over consumption (requiring storage over extended periods) coupled with high relative humidity, farmers in Mutasa preferred cultivars with grain weevil resistance to those with drought stress tolerance. In addition, the high relative humidity and rainfall in Mutasa could also explain why these farmers preferred pest and disease resistance ahead of abiotic stress tolerance. Tolerance to low soil fertility would be equally important in Mutasa, because the high rainfall would result in leaching of nutrients from their soils. The leaching of soil nutrients would not be a major problem in the severely drought-prone Chipinge and Mutare West. Alternatively, tolerance to low soil fertility would not be the priority because without water, the plants cannot take up nutrients even when they are sufficiently available in

the soil. Thus, farmers in Mutare West showed their strong preference for drought stress tolerance ahead of even high yield potential.

Results indicated that farmers, especially in Mutasa and Chipinge, preferred a cultivar that would combine high yield potential and early maturity. The ultra short season (less than 90 days) in these areas indicated that early maturing cultivars with tolerance to drought stress at flowering would be suggested for deployment in Chipinge and Mutare West. Farmers' quest for drought tolerant maize with a stress recovery mechanism similar to that of sorghum should be taken seriously. Apart from sorghum's ability to produce extra tillers when rainfall resumes, it is suggested that there may be some genes that confer higher drought tolerance in sorghum than maize. Studies of locally adapted sorghum cultivars should be conducted to investigate the drought stress recovery mechanism, with a possibility of transferring the genes into locally adapted maize cultivars. In the absence of truly drought tolerant maize, farmers may be encouraged to grow more sorghum, especially in Chipinge as an interim measure. In Mutare West, farmers indicated that they grew more sorghum than maize; hence it would also be important to consider improving their sorghum cultivars. However, in the long term drought tolerant maize cultivars should be bred because farmers indicated strong desire to grow maize.

Apparently, farmers showed low concern about pounding ability and good husk cover, which contrasted sharply with previous studies in other areas. Banziger and de Meyer (2002) reported that farmers would prefer hard endosperm types for ease of pounding and good husk cover for protection against storage pests and ear rots. The first contrast may be explained by the increased use of hammer mills, while the second by the relatively limited surplus grain demanding little or no storage at all. However, farmers' preference for early maturing cultivars was consistent with previous studies. Banziger and de Meyer (2002) reported that farmers would prefer early maturing cultivars because they can escape the late season drought. Farmers' preference for long and thin cobs compared to long and fat cobs was rather difficult to explain. Scientists would expect farmers to have strong preference for long and fat cobs. However, their preference for semi-dent and flintier grain texture was consistent with previous studies in other areas of southern Africa (Banziger and de Meyer (2002), but differed from studies in Kenya (De Groote, 2000). De Groote (2000) reported that farmers in eastern Kenya were not interested in flintiness and cob length. This shows that farmers' preferences are peculiar to their area and depend on the major prevailing constraint.

2.5   Conclusions and Implications for Breeding

This study examined farmers' perceptions on maize cultivars, production constraints, and preferences for stress tolerant cultivars, and their implications for breeding. The results showed that given the different agro-ecologies, maize production constraints differed among survey districts influencing farmers' preferences for maize cultivars and traits. For instance, farmers in more productive areas with the potential of producing surplus grain strongly preferred weevil-resistant cultivars, whereas those in less productive areas preferred drought tolerant cultivars.

It was shown that farmers had strong preference for hybrids of the 1970's as they were perceived to be more resilient to drought than new hybrids. This suggested that no progress has been made over the past 35 years in breeding cultivars with drought tolerance for deployment in these marginal areas. A cultivar combining high yield potential, early maturity and drought tolerance was generally preferred in all areas. An ultra early maturing cultivar with drought tolerance, especially at flowering could be suggested for deployment in Chipinge and Mutare West. Farmers' quest for drought tolerant maize with a stress recovery mechanism similar to that of sorghum, suggested that genes conferring drought tolerance in local sorghum should be investigated. The study also showed that cultivars tolerant to low soil fertility, pests and diseases would be desirable for release in high rainfall areas where nutrient leaching and pest and disease epidemics would be a problem. In addition, the local land-race "Chitonga" should be improved by selecting for short plant height and short maturity period, without compromising its sweetness and flint grain texture. This would improve households' food security and the livelihoods of resource poor farmers in Chipinge and Chimanimani area, with possible spillover effects into the neighbouring Mozambique. In summary, results implied the need to integrate conventional and participatory or interactive strategies for clear identification of farmers' preferences for cultivars that are adaptable, high yielding and stress tolerant, among other traits, in these marginal districts.

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Appendices

Appendix 1: Sample study area and number of respondents in the survey

District	Area or Village	Ecological Zone	Long term Annual Rainfall (mm)	Number of Households
Pilot Survey Study				
Mutasa	Honde Valley	Region II	700-1050 16-18 wet pentads♠	**6**
Chipinge	Birchenough Bridge	Region IV	450-500 <14 wet pentads	6
Mutare West	Marange	Region III	650-800 14-16 wet pentads	3
Informal Focus Group Discussion				
Chipinge				**29**
	Kondo Village	Region IV	450-500	12
	Nyakumanwa	Region IV	<14 wet pentads	17
Chimanimani				**9**
	Changazi			9
Formal Household Survey				
Chipinge				**37**
	Masocha	Region III	650-800	9
	Taozeni		14-16 wet pentads	7
	Kondo	Region IV	450-500	14
	Musapingura		<14 wet pentads	8
Mutasa				**25**
	Sadziwa	Region IIb	700-1050	9
	Tadyanemhandu		16-18 wet pentads	8
	Musakwa			8
Mutare West (Marange)	Mafararikwa	Region III	650-800	**31** 6
	Sendamurambi		14-16 wet pentads	6
	Mushipe			6
	Mutsago			6
	Masase			7
Total Sample				**146**

Appendix 2: Farm economy and household characteristics in sample districts

Characteristic	District			Overall Mean	Statistic: (F. probability)
	Mutasa (n=25)	Chipinge (n=37)	Mutare West (n=31)		
Number in Household					
Male Adults	1.0	0.9	1.6	1.2	0.01
Female Adults	1.2	1.4	2.0	1.6	0.01
Male Children	2.5	2.4	3.6	2.9	0.35
Female Children	2.6	2.8	3.3	2.9	0.65
Total Household Size	7.3	7.5	10.5	8.5	-
a) Number of farm livestock per household					
Cattle	7.6	4.5	4.8	5.5	0.02
Chicken	10.9	10.5	9.0	10.1	0.47
Goats	3.4	4.8	6.7	5.1	0.03
Sheep	0.3	1.7	0.0	0.6	0.01
Donkeys	0.0	0.6	0.1	0.2	0.00
Pigs	0.0	0.0	0.0	0.0	0.31
b) Farm tools or physical stock					
Tractor	0.0	0.1	0.0	0.0	0.32
Cart	0.8	0.5	0.5	0.6	0.04
Plough	0.9	0.8	1.1	1.0	0.08
Harrow	0.6	0.2	0.0	0.2	0.00
Well	0.5	0.5	0.7	0.6	0.31
Pump	0.0	0.0	0.0	0.0	-
c) Household amenities					
Modern House	1.6	1.2	1.3	1.3	0.18
Traditional hut	1.6	2.2	2.2	2.0	0.04
Motor Vehicle	0.1	0.1	0.0	0.1	0.56
Television set	0.2	0.0	0.0	0.1	0.00
Radio	1.1	1.1	1.1	1.1	0.98
d) Land holding (ha) and crops grown					
Maize	1.0	1.6	0.5	1.0	0.00
Bean	0.1	0.0	0.1	0.1	0.00
Sorghum	0.0	0.4	0.8	0.5	0.00
Groundnuts	0.2	0.2	0.2	0.2	0.31
Pearl-millet	0.0	0.0	0.1	0.0	0.00
Total Land holding	1.3	2.2	1.7	1.7	-

Appendix 3: Farmers' perception for soil fertility, rainfall and drought (mean scores)

Characteristic [*]	Mutasa	Chipinge	Mutare West	Across Districts	Statistics F. Probability
Soil Characteristics					
Texture	2.0	2.4	1.8	2.1	0.00
Fertility	1.9	1.6	2.1	1.9	0.00
Soil Fertility Management					
Manure Applied (kg/ha)					
Cattle Manure	1300	1125	1148	1196	0.84
Chicken Manure	75	87	0.0	53	0.00
Goat Manure	68	38	19	38	0.16
Mean Rank of Productivity of Manure					
Cattle Manure	1.3	1.2	1.4	1.3	0.29
Chicken Manure	1.5	1.9	2.1	1.9	0.04
Goat Manure	2.7	2.7	2.3	2.5	0.17
Application of Inorganic fertiliser (kg/ha)					
Basal Fertiliser	225	250	112	188	0.01
Top Dressing	188	188	225	150	0.29
Amount of Rain					
2004	2.0	1.1	2.0	1.7	0.00
2003	2.4	1.6	2.0	2.0	0.00
2002	2.7	1.3	2.1	2.0	0.00
Nature of Drought					
	3.0	2.3	2.3	2.5	0.00
Intensity of Drought					
	2.1	2.9	2.9	2.7	0.00
Date of first Rainfall					
2004	2.0	2.8	1.8	2.2	0.00
2003	2.0	1.4	2.0	1.8	0.00
2002	2.8	1.4	2.0	2.1	0.00
Date of last Rainfall					
2004	2.1	1.4	1.0	1.5	0.00
2003	2.8	2.6	1.1	2.1	0.00
2002	2.0	2.4	1.2	1.9	0.00

[*] **Fertility**: 1 = Good, 2 = Moderate, 3= Low; Texture: 1 = Sand, 2 =Clay, 3 =Loam; **Rainfall amount**: 1= little, 2= moderate, 3= sufficient for maize crop; **Nature of Drought**: 1=early, 2= mid, 3=late, 4 = whole season drought; **Intensity of Drought**: 1 = little, 2 = moderate, 3 = severe; **Date of first Rain**: 1 = late Oct, 2 = Nov, 3 = Dec and   4 = Jan; **Date of last Rain**: 1 = Feb, 2 = March, 3 = April 4 = May.

Appendix 4: Maize cultivars or brands grown by farmers in the sample districts

Cultivar	Districts			Overall
	Mutasa	Chipinge	Mutare West	
	(% Farmers indicating they grew the cultivar)			
SC500 BRAND♠	0	0	87	32
SC513	40	48	6	30
SC401	36	0	3	12
PAN 6479	0	22	0	7
SC400 BRAND♠	4	4	13	7
SC403	0	15	0	6
Farm Saved Seed	16	4	0	6
PANNAR BRAND♠	8	0	10	6
PAN 413	0	15	0	5
SC601	12	0	0	4
SC501	8	0	0	2
SC701	4	0	0	1
Pioneer Brand♠	0	0	3	1

♠Farmers recognised the brand but not specific hybrid name.

Chapter 3: Genetic Analysis of Resistance to Gray Leaf Spot Disease in Southern African Maize Germplasm

Abstract

Production of maize in Southern Africa is threatened by the yield limiting gray leaf spot disease (*Cercospora zeae-maydis* Tehon and Daniels) among other constraints, yet there is limited knowledge of the genetic effects conditioning resistance in Southern African base germplasm. This study was conducted to: (a) evaluate resistance; (b) determine heritability; (c) assess combining ability of inbred lines; and (d) investigate the mode of gene action conditioning resistance to gray leaf spot (GLS) in Southern African base germplasm. Twenty-seven inbred lines were mated in sets according to a North Carolina Design II scheme. The resultant 72 hybrids, 27 inbred lines and 24 standard hybrids were evaluated in replications of an 8 x 12 α-lattice design, in four environments in Zimbabwe and South Africa during 2004/5. Results revealed highly significant (P<0.01) environment, hybrid and hybrid x environment interaction effects for GLS and yield. Resistance was highly heritable (72%) and highly predictable. General combining ability (GCA) and specific combining ability (SCA) effects were highly significant (P<0.01), indicating that both additive and non-additive effects were important in conditioning resistance. General combining ability effects accounted for 86% of cross sum of squares for GLS, indicating that additive effects were predominant in controlling resistance. Hybrids between resistant and susceptible (R x S and S x R) inbred lines showed high levels of resistance, suggesting that dominance also played a role in conferring resistance. Thus, it was suggested that single cross hybrids would display adequate resistance relative, when at least one inbred line is resistant to GLS. Large differences between male and female GCA mean squares provided the basis for the speculative role of maternal effects in influencing resistance. Future studies should use models that incorporate reciprocal effects to confirm the role of maternal effects. The following hybrids, which displayed high yield potential and high resistance to GLS, would be recommended for release: A9/A15 (relative yield to overall mean = 109%), A13/B19 (117%), A7/A15 (113%), A15/B21 (116%), B24/B18 (117%), A9/A13 (119%) and B19/CML444 (111%). The inbred lines A13, A15, B18 and B19 showed significant (P<0.05) negative GCA as both male and female sources and contributed high levels of resistance to their hybrids. These lines would be recommended for use as resistance sources.

Keywords: Gene Action, Gray Leaf Spot, Heritability, Maize, Resistance,

3.1 Introduction

Small-scale farmers in sub-Saharan Africa dominate production of maize, which is unfortunately threatened by endemic and highly yield limiting diseases such as gray leaf spot (*Cercospora zeae-maydis* Tehon and Daniels). Gray leaf spot disease (GLS) has during the past 17 years spread from South Africa, Zimbabwe, Uganda, and Kenya to West Africa in areas where maize is grown under warm and humid conditions (Menkir and Ayodele, 2005; Caldwell *et al*., 2002; Pixley, 1997). Gray leaf spot causes direct damage of photosynthetic tissue and indirect damage in the form of stalk and root lodging. This damage forms the basis for the grain yield loss in susceptible maize cultivars. Scientists have reported varying levels of loss that is attributable to GLS in temperate and tropical maize. Munkvold *et al*. (2001) cited huge economic loss of $100 million in Iowa, and yield reduction of 24 to 69% (Virginia), 11 to 44% (Iowa) and 29 to 65% (South Africa). Cromley *et al*. (2002) cited a 10 to 25% yield reduction in susceptible temperate hybrids, which was consistent with 28% previously reported during fungicide spraying trials (Stromberg and Donahue, 1986). Ward *et al* (1999) reported 11 to 69% in South Africa, whereas Menkir and Ayodele (2005) cited that extreme losses reaching 100% could occur under severe epidemics. Average loss seems to revolve around 20% as cited by many scientists in the USA (Huff *et al*., 1988; Elwinger *et al*., 1990; Thompson *et al*., 1987; Donahue *et al*., 1991). Thus, the disease poses a threat to food security in Southern Africa.

Scientists, especially in the USA, have devoted great effort in studying genetics of GLS resistance. These studies could be used in making decisions when breeding for resistance to GLS. Many studies have concluded that additive effects have a larger role than non-additive effects in conditioning resistance (Menkir and Ayodele, 2005; Cromley *et al*., 2002; Ulrich *et al*., 1990). A survey of literature has even identified cases in which resistance was explained by the GCA (additive) effects alone (Bubeck *et al*., 1993; Ulrich *et al*., 1990; Thompson *et al*., 1987). There is no single mention of situations where SCA (non-additive) was larger than GCA effects. Generally, it has been reported that resistance involved five or six genes acting in an additive fashion (Clements *et al*., 2000). Contributions of dominance to conferring resistance have also been reported in both temperate and tropical African germplasm (Clements *et al*., 2000; Coates and White, 1998; Hohls *et al*., 1995).

Despite the fact that the disease is now endemic, there is still limited literature about genetic analyses of resistance in Southern African maize base germplasm. Early

studies in South Africa (Gevers *et al*., 1994; Hohls *et al*., 1995) used materials that are not easily adaptable to the more tropical northern part of the continent. In addition, a lot of new germplasm has been bred, which has to be evaluated and the information shared with other regional scientists. There is still a lot of reliance on the information generated from the USA, which has limited application to the humid tropical zones in the region. The objective of the current study was therefore, to: (a) evaluate resistance level; (b) determine heritability; (c) assess combining ability of inbred lines; and (d) investigate the mode of gene action conditioning resistance to GLS in a set of Southern African maize base germplasm.

3.2   Materials and Methods

3.2.1 Germplasm

The inbred lines used in the study were drawn from the following heterotic groups: a) International Maize and Wheat Improvement Centre (CIMMYT) A and B, and b) regional: I, P, K, SC, N3, M and their derivatives (Table 3.1). Gevers and Whythe (1987) and Mickelson *et al*. (2001) have presented detailed descriptions of these germplasm groups. The inbred lines consisted of six GLS resistant lines, six drought tolerant lines and 15 conventional lines obtained from CIMMYT and Seed Co in Zimbabwe. Conventional lines had not been bred for resistance to GLS or drought stress tolerance, but were selected on the basis of high yield potential. However, all the inbred lines were adapted to tropical environments in east and Southern Africa. Inbred lines were divided into sets of three inbreds each, according to their resistance status. In making crosses, three inbred lines in one set were used as females and crossed with three inbred lines from another set used as males, according to a North Carolina Design II Mating Scheme (Robinson and Comstock, 1952; Hallauer and Miranda, 1988). Each inbred line was used once as a female parent in one set and once as a male parent in another set, except the inbreds K64R, B11 and B12, which were used as females only, and CML489, A26 and CIM24, which were used as male parents only (Appendix 1, in sets 8 and 4, respectively). The inbreds CML489, CIM24 and A26 replaced K64R, B11 and B12 as male parents in set 4, which had failed to provide adequate pollen due to severe attack by maize streak virus. Consequently, eight sets comprising nine hybrids each were formed among the 27 inbreds. Lines within a set had similar resistance levels; hence the resultant hybrids were crosses between susceptible and susceptible (S X S); resistant and resistant (R X R) and between resistant and susceptible (R X S and S X R) inbred lines. In total, 72 single cross F_1 generation hybrids (8 sets x 9 hybrids each) were made

among the inbred lines (Appendix 1). The reference population for the study was effectively constituted by the 27 parent inbred lines, their 72 crosses and 24 standard hybrids. Among the 24 standard hybrids SC627 and SC513 were used as GLS resistant check hybrids, while SC403, SC633, PAN6777, ZS255, ZS257, R201 and R215 were widely grown hybrids throughout Southern Africa.

Table 3.1: Parent Inbred Lines used in a NC Design II Mating Scheme

Inbred	Designation	Heterotic Group	Principal Selection Criteria
1	CML442	A	Drought Tolerance
2	CML312	A	Drought Tolerance
3	CML445	AB	Drought Tolerance
4	CML395	B	Drought Tolerance
5	CML444	B	Drought Tolerance
6	CML488	B	Drought Tolerance
7	A7	M	Conventional[*]
8	A8	M	Conventional
9	A9	P	Conventional
10	K64R	K	Conventional
11	B11	K	Conventional
12	B12	K	Conventional
13	A13	A	Gray Leaf Spot Resistance
14	A14	A	Gray Leaf Spot Resistance
15	A15	N	Gray Leaf Spot Resistance
16	B16	I	Gray Leaf Spot Resistance
17	B17	B	Gray Leaf Spot Resistance
18	B18	B	Gray Leaf Spot Resistance
19	B19	K	Conventional
20	B20	KB	Conventional
21	B21	K	Conventional
22	B22	S	Conventional
23	B23	S	Conventional
24	B24	S	Conventional
25	CML489	AB	Conventional
26	A26	I	Conventional
27	CIM24	A	Drought Tolerance

[*]Conventional inbreds were not bred for stress tolerance, but were bred for high yield potential.

3.2.2 Experimental Design

Inbred parents were evaluated in a 5 x 6 α-lattice design with three replications at Rattray Arnold Research Station (RARS; 1341 m altitude) in Zimbabwe, in 2004/5 season. In addition to RARS, hybrids were evaluated at Cedara Agricultural Institute (CED; 1076 m altitude) in South Africa and at the Agricultural Research Trust farm (ART; 1527 m altitude) in Zimbabwe. In 2004/5 seasons, 96 hybrids consisting of 72 experimental and 24 hybrid checks were evaluated in a 12 X 8 α-lattice block design with two replications at Cedara, RARS and ART. At the experimental sites fertiliser (kg/ha) was applied as follows: 120 N: 33P: 44K at Cedara; 208N: 35P: 21K at RARS; and 250N: 65P: 25K at ART. Phosphorus (P) and potassium (K) were applied once as basal at planting, but additional nitrogen (N) was applied in two equal splits at five weeks

from crop emergence and anthesis. Total precipitation was 885 mm at Cedara (2004/5); 787 mm at ART (2004/5) and 826 at RARS (2004/5). The established plant populations (plants/ha) were 44 000 at Cedara and 53000 at RARS and 53000 at ART. Standard cultural practices which included hand planting, hand weeding and application of herbicides were followed at all sites. Fields were left to natural disease inoculation and disease development was monitored every fortnight beginning from tassel emergence.

Gray leaf spot disease severity was assessed at mid silking (GLS1) and at hard dough stages (GLS2), based on visual assessment of the whole plot by estimating: (a) percentage leaf area diseased or necrotic (% LAD); and (b) using a rating scale of 1 to 9. Using this scale, 1 = no disease symptoms on leaves, 3 = lesions are on lower leaves and no lesions disease on leaves above the ear, 5 = disease is on most leaves and some lower leaves dead, 7 = dead lowerleaves and many lesions on all leaves above the ear, and 9 = nearly all the leaves are dead (Munkvold et al., 2001). The % LAD and rating scale were used to evaluate the disease at Cedara, while only the rating scale was used at RARS and ART in 2004/5 season. At harvest grain yield was measured on a whole plot basis following standard practice used at CIMMYT (CIMMYT, 1985) and was adjusted to 12.5% moisture using the formula:

Grain Yield (t/ha) = [Grain Weight (kg/plot) x 10 x (100-MC)/ (100-12.5)/(Plot Area)], where MC = Grain Moisture Content.

The number of days to mid silking (DMS) and anthesis (DMP) were estimated as number of days from planting to 50% plants with silk emerged and tassels shedding pollen, respectively. Plant and ear height were measured as the distance from the base of the plant to the height of the first tassel branch and the node bearing the uppermost ear, respectively. Grain texture was rated using a scale of 1 to 5, which is used at CIMMYT, where 1 = flint and 5 = dent. In addition to grain texture analysis, ear aspect was assessed using a visual rating scale of 1 to 9, where 1= clean, uniform, large and well-filled ears, and 9 = rotten, variable, small and partially filled ears.

Mid parent heterosis (MPH) for GLS was calculated for each hybrid (Falconer, 1989) using the formula:

MPH (%) = $(F_1-MP)/MP$ X 100,

Where F_1 = mean of the F_1 hybrid performance, MP = mean of two parents making the cross using the formula: $(P_1 + P_2)/2$, where P_1 and P_2 are the means of the inbred parents.

3.2.2 Statistical Analyses

General analyses of variance were performed for all hybrids and inbred lines including controls, using IRRISTAT (2003) computer package for GLS and grain yield data for each individual site. Genetic analyses for GLS were performed in SAS (SAS Institute, 1997) as a fixed effects model for experimental hybrids only, as described by Hallauer and Miranda (1988) for across environments (RARS, Cedara and ART in 2004/5), which had complete sets of hybrids made in the North Carolina II mating design, using the following linear model:

$$Y_{ijkpq} = \mu + S_p + g_i(S_p) + g_j(S_p) + h_{ij}(S_p) + E_q + r_k(SE)_{pq} + (ES)_{pq} + (Eg)_{iq}(S_p) + (Eg)_{jq}(S_p) + (Eh)_{ijq}(S_p) + e_{ijkpq}$$

Where i =1, 2, 3; j = 1, 2, 3; k = 1, 2; p = 1, 2, 3, 4, 5, 6, 7, 8; q = 1, 2,3 and Y_{ijkpq} denotes the value of the hybrid of a mating of the ith female line, the jth male line, in the kth block, within set p and in the qth environment. The terms are defined as follows:

μ = Grand mean,

S_p = the average effect of the pth set,

$g_i(S_p)$ = the GCA effect common to all hybrids of the ith female line nested within p^{th} set,

$g_j(S_p)$ = the GCA effect common to all hybrids of the jth male line nested within p^{th} set,

$h_{ij}(S_p)$ = the SCA effect specific to hybrid of the ith female and j^{th} male line nested within p^{th} set,

E_q = average effect of qth environment,

$r_k(SE)_{pq}$ = the effect of the kth replication nested within the pth set and q^{th} environment

$(ES)_{pq}$ = the interaction between set effects and the environment

$(Eg)_{iq}(S_p)$ and $+(Eg)_{jq}(S_p)$ = the interaction between environment and GCA nested within sets

$(Eh)_{ijq}(S_p)$ = the interaction between environment and SCA nested within sets, and

e_{ijkpq} = the random experimental error.

According to Hallauer and Miranda (1988), the main effects due to females (sets) and males (sets) are equivalent to the GCA (general combining ability), while male x female (sets) interaction effects represent the SCA (specific combining ability) effects in a diallel mating. General combining ability and SCA effect estimates for inbred lines and crosses, respectively, were determined by line x tester analysis in Agrobase (2005) computer package. Using the variance ratios, heritability estimates were calculated in REML (GenStat, 2003) as suggested by Hallauer and Miranda (1988) for the fully inbred parents (F = 1) (where $\sigma^2_m = \sigma^2_f = \frac{1}{2}\sigma^2_A$; and $\sigma^2_{mf} = \sigma^2_D$) using the formulae:

a) $h^2 = 2\sigma^2_m/(\sigma^2/r + \sigma^2_{mf} + 2\sigma^2_m)$ for one environment, and

b) $h^2 = 2\sigma^2_m/(\sigma^2/re + \sigma^2_{fme}/e + 2\sigma^2_{me}/e + \sigma^2_{mf} + 2\sigma^2_m)$ for across environments,

Where σ^2_m = male (set) variance, σ^2 = random error variance; σ^2_{mf} = male x female (set) variance; σ^2_{fme} = environment x male x female (set) variance; σ^2_{me} = environment x male (set) variance; r = number of replications and e = number of environments. σ^2_f = female (set) variance; σ^2_A = additive variance and σ^2_D = dominance variance.

Heritability estimates were calculated using the male variance (σ^2_m) to avoid the upward bias of heritability due to maternal effects if the female variance (σ^2_f) was used. Predictability of hybrid performance was also measured by performing a regression of hybrid means on mid-parent values for GLS and grain yield, using GenStat (2003) computer software. Correlation analyses were performed between GLS assessment methods; and between hybrid rankings in different environments.

3.3   Results

3.3.1 Resistance of Inbred Lines

Inbreds exhibited significant variation for resistance and yield during 2004/5 at RARS (Table 3.2). Severity of GLS disease was low at RARS (Table 3.2). The most susceptible inbred line had a rating of 5.5 (CML312), but all inbred lines had GLS symptoms. Thus, GLS scores ranged from 1.1 to 5.5 (Table 3.2). For the convenience, inbred lines could be fitted into three resistance classes as follows: a) resistant (GLS score = 1 to 2.0); (b) moderately resistant/susceptible (2.1 to 3.9); and c) susceptible (4.0 to 5.5). Resistant inbreds had GLS scores that were significantly below the mean. The inbreds in the moderately resistant or susceptible category had GLS scores that were similar to the mean. The susceptible class consisted of inbreds with GLS scores significantly above the mean. Nine inbred lines were resistant to GLS. The inbreds A14, B16 and B17, which had been selected as resistant based on pedigree data were among the susceptible (Table 3.2). The inbreds had significant (P≤0.05) differences for grain yield. Five of the resistant inbred lines namely, B18, B19, A15, A13 and CIM24 had above average yield (relative yield ≥100%), while four of the resistant inbreds had below average yield (Table 3.2). The correlation between GLS resistance and grain yield was not significant (r = 0.21). The highest yielding inbred lines (CML489 and CML444) were in the moderately resistant/susceptible class.

Table 3.2: Performance of 27 Inbred Lines at RARS during 2004/5 season

Inbred	GLS		Yield	
	Category[*]	(Score)	(t/ha)	% Mean
B22	R	1.1	1.2	55
B24	R	1.1	0.9	40
CIM24	R	1.5	2.4	111
A13	R	1.6	2.2	100
A15	R	1.6	2.4	109
9301485	R	1.7	1.4	64
B23	R	1.7	1.6	71
B18	R	1.9	2.5	113
B19	R	2.0	2.4	111
CML489	MS	2.3	3.0	137
A8	MS	2.5	2.8	126
CML488	MS	2.9	2.2	102
B21	MS	3.0	2.2	99
CML445	MS	3.0	2.8	127
CML442	MS	3.1	2.4	110
A14	MS	3.3	2.3	105
CML444	S	4.0	3.0	138
K64R	S	4.0	1.5	68
CML395	S	4.2	3.4	157
RSA414P	S	4.2	1.0	46
A9	S	4.3	2.3	105
A7	S	4.4	2.8	127
B16	S	4.5	1.8	82
B17	S	4.6	2.6	119
B20	S	4.7	2.5	112
A26	S	4.8	1.7	79
CML312	S	5.5	2.6	117
Mean		2.93	2.21	100
SED		0.33	0.20	
LSD (0.05)		0.95	0.58	

[*]R= resistant, S = Susceptible, and MS = moderately susceptible; SED = Standard error of a difference

3.3.2 Hybrids' Resistance

The data from three environments that had a complete set of experimental hybrids in 2004/5 are presented (Table 3.3). There were significant differences among hybrids within and across environments for GLS-resistance and grain yield (Table 3.3). The GLS scores ranged from 1.2 to 7.0 for the hybrids A9/A15 and ZS206, respectively (Table 3.3). The highest disease pressure occurred at Cedara, where the scores ranged from 1.5 for A9/A15 to 9.0 for standard hybrids ZS206, SC701 and R215. The most resistant hybrids had scores of 1.0 at RARS and ART, but disease severity was lowest at ART where it ranged from 1.0 (no symptoms) to 5.0 (Table 3.3). The 96 hybrids were fitted into resistance classes using the data obtained from Cedara, which showed the highest disease incidence (Fig. 1). The data from Cedara alone was used to classify hybrids because it provided the best discrimination of hybrids for GLS-resistance (Table 3.3). Unlike at ART and RARS, there were no hybrids that were rated as immune (GLS score = 1.0) at Cedara in 2004/5. The four resistance classes consisted of: a) resistant (GLS score = 1.6 to 2.6); b) moderate resistance (GLS score = 2.7 to 4.7); c) moderately susceptible (GLS score = 4.8 to 6.8); and d) susceptible (GLS score = 6.9 to 8.9). Hybrids in the susceptible and resistant classes had GLS scores that were significantly different from the mean score. Sixteen experimental hybrids were classified as resistant and had higher levels of resistance comparable to resistant standard checks. A similar set of hybrids dominated the top 10 ranking across environments. The standard hybrids SC513 and SC627 were resistant, while ZS255, SC633 and SC635 were moderately resistant to GLS (Table 3.3). The standard hybrids R201, ZS206, SC701 and R215 were highly susceptible with GLS scores of 8.5 to 9.0 at Cedara. The most resistant hybrids showed high relative yield potential at Cedara (>109%). The following hybrids displayed high yield potential and high resistance to GLS: A9/A15, A13/B19, A7/A15, A15/B21 and B24/B18 (Table 3.3).

Table 3.3: GLS Resistance and Relative Yield for Selected Hybrids in 2004/5

Hybrid[♣]	Across Sites				ART		CED		RARS	
	GLS		Yield		GLS	Yield	GLS	Yield	GLS	Yield
	(Score)	Rank	% Mean	Rank	(Score)	% Mean	(Score)	% Mean	(Score)	% Mean
Top 15 GLS- resistant hybrids										
A9/A15	1.2	1	**106**	32	1.0	**110**	1.5	**109**	1.0	101
A13/B19	1.2	2	96	63	1.0	83	1.5	**117**	1.0	94
A7/A15	1.3	3	**115**	10	1.0	103	2.0	**113**	1.0	**128**
A15/B21	1.3	4	**111**	15	1.0	**119**	2.0	**116**	1.0	99
B24/B18	1.3	5	**108**	21	1.0	99	2.0	**117**	1.0	**111**
B18/CML442	1.3	6	101	47	1.0	96	2.0	98	1.0	**107**
SC513	1.3	7	93	72	1.0	96	2.0	91	1.0	92
B23/B18	1.3	8	90	79	1.0	84	2.0	88	1.0	98
B22/B18	1.3	9	72	95	1.0	82	2.0	74	1.0	62
A9/A13	1.5	10	101	44	1.0	99	2.0	**119**	1.5	91
04C1679	1.5	11	100	51	1.0	102	2.0	103	1.5	97
B19/CML444	1.8	16	**120**	5	1.0	**128**	2.0	**111**	2.5	**117**
SC627	2.2	24	**106**	30	1.0	**110**	2.0	91	3.5	**115**
04C1675	1.5	12	68	96	1.0	81	2.5	54	1.0	65
A8/A13	1.7	14	99	54	1.0	**107**	2.5	97	1.5	93
Middle 10 or Moderately resistant										
CML395/CML489	2.7	36	**112**	14	1.0	**115**	4.5	104	2.5	**114**
ZS255	2.8	44	75	93	1.5	70	4.5	85	2.5	74
SC633	3.3	49	**109**	18	2.5	91	4.5	**121**	3.0	**119**
B11/B24	3.4	52	75	94	2.2	101	4.5	64	3.5	58
A15/B20	3.5	55	84	86	2.5	101	4.5	81	3.5	70
A15/B19	2.3	32	96	62	1.0	100	5.0	99	1.0	91
B19/CML488	2.7	38	**107**	28	1.5	103	5.0	**124**	1.5	98
SC635	3.0	45	**108**	24	1.0	102	5.0	98	3.0	**121**
B17/CML445	3.5	53	**109**	17	1.5	107	5.0	**105**	4.0	**115**
CML488/CML489	3.5	54	95	67	2.5	82	5.0	**107**	3.0	99
Bottom 9 GLS-susceptible										
B16/CML442	5.0	83	102	41	3.5	88	7.5	**123**	4.0	100
B23/B17	5.2	86	102	42	4.0	101	7.5	**106**	4.0	100
CML442/A9	5.7	90	**109**	19	3.5	**106**	7.5	**106**	6.0	**113**
K64R/B23	5.7	92	79	89	3.5	74	8.0	74	5.5	87
A7	5.7	91	94	71	3.5	99	8.5	83	5.0	98
R 201	7.0	96	77	91	5.0	76	8.5	75	7.5	78
ZS206	7.0	93	96	65	5.0	**106**	9.0	89	7.0	91
SC701	7.0	94	94	70	5.0	102	9.0	78	7.0	99
R 215	7.0	95	80	88	5.0	83	9.0	75	7.0	81
Mean	3.36	100			2.21	100	4.75	100	3.12	100
SED					0.40		0.70		0.40	
LSD (5%)					1.10		2.00		1.20	

[♣]Standard hybrids and high yield values are in bold; Relative yield = % of overall mean yield.
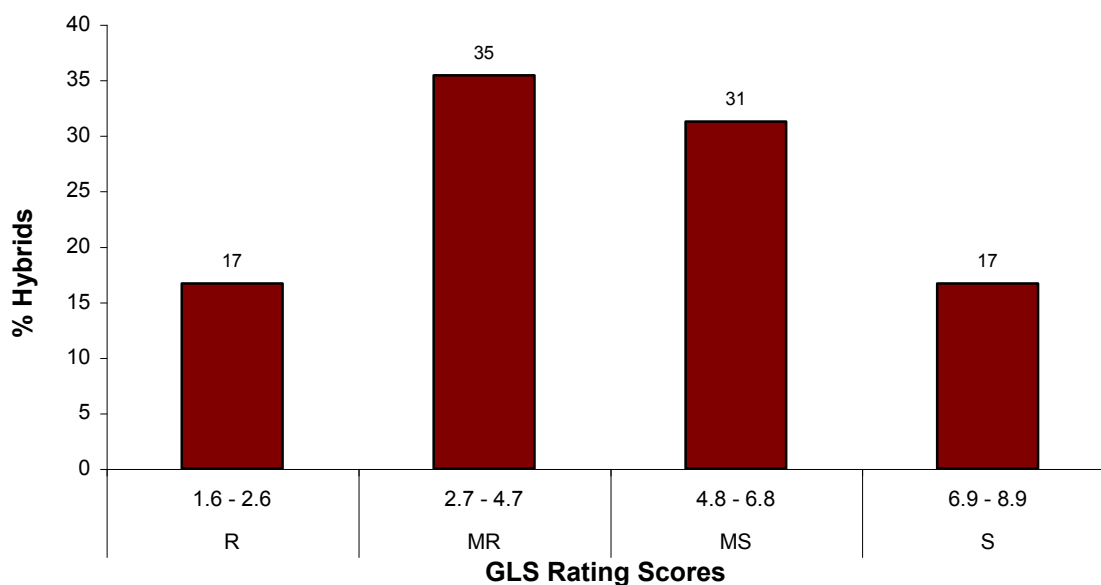
Fig. 1: Distribution of 96 Hybrids for Resistance to GLS at Cedara in
2004/5 season (R = resistant, S = susceptible, M = moderate)

3.3.3 Relationship between GLS Rating and Grain Yield

Results showed highly significant and positive correlation between the first and second GLS assessments for both GLS rating scores and %LAD (Table 3.4). There were also highly significant ($P \leq 0.01$) positive rank correlations between sites for hybrid GLS rating scores and % LAD (Table 3.4). However, there was no significant correlation between grain yield and GLS resistance within (r = -0.12 to 0.18) and across (r = -0.13) sites for either hybrids or parent inbred lines.

Table 3.4: Correlation between GLS assessments and between sites for hybrid
ranking during 2004/5

Between Rating Methods	LAD1 (%)	LAD2 (%)	GLS1 Score
LAD1 (%)	1.00	0.77	
LAD2 (%)	0.77	1.00	
GLS1 Score	0.79	0.79	1.00
GLS2 Score	0.74	0.96	0.78
Between Environments	CED	RARS	
CED	1.00	0.79	
ART	0.84	0.80	

3.3.4 Gene Action

There were significant differences among hybrids for grain yield and GLS rating scores (Table 3.5). Environment and sets of hybrids and the environment x sets interaction were significant for grain yield and GLS rating (Table 3.5). Both male (set) and female (set) main effects were significant for yield and GLS rating score (P≤0.01), with mean squares for females (sets) larger than their male (sets) counterparts for grain yield. Mean squares for males (sets) were larger than those for females (sets) for GLS scores. Interaction of female and male within sets was highly significant (P≤0.01) for grain yield, and GLS. Female (sets) interaction with environments was not significant for GLS. Interaction of male x female (sets) with environments was only significant for GLS. Total GCA (i.e., male plus female main effects) accounted for 74% and 86% of cross sum of squares for grain yield and GLS, respectively. Female lines accounted for 33% and males 53% of the total GCA sum of squares for GLS. For yield, female lines contributed 54% and male lines 20% of the total GCA sum of squares.

Table 3.5. Mean square values for grain yield and GLS scores across three environments

Source of Variation	D.F.	Yield	GLS
Environment	2	232.8**	248.0 **
Set	7	13.9**	17.7 **
Environment x Set	14	3.1**	2.0 **
Replication (Set) (Environment)	24	1.2 ns	0.6 ns
Female (Set)	16	7.4**	13.4**
Male (set)	16	2.6**	21.3 **
Female x Male (Set)	31	1.8**	2.9 **
Environment x Female (Set)	32	1.6 *	0.7 ns
Environment x Male (set)	32	1.9**	2.4 **
Environment x Female x Male (Set)	62	1.0 ns	1.2 **

**, * = Significant at P< 0.01 and P< 0.05, respectively; ns = non-significant at P>0.05.

3.3.5 Combining Ability Effects of Inbred Lines

Among the resistant inbreds lines (Table 3.2) A13, A15, B18 and B19 exhibited significantly negative GCA effects as both male and female sources for resistance (Table 3.6). The lines B24 and B12 had significant negative GCA as male and female sources, respectively, of resistance. Among the susceptible set (Table 3.2), the inbred A9 displayed significant (P≤0.05) negative GCA as a female source for both GLS scores and % LAD. As expected all the other susceptible lines had either significant positive (P≤0.05) or zero GCA for GLS. Nonetheless, only B23 did not show significant (P≥0.05)

GCA among the resistant lines, while B22 only displayed significant GCA effects as male source for % LAD. Inbreds A9 and A13 combined both positive GCA for yield and negative GCA for GLS as female sources only. A highly susceptible inbred, A26 had highly positive (P≤0.01) GCA for both grain yield and GLS resistance. On the contrary, some lines (B12, A15, B19 and CIM24) that lacked significant positive GCA for yield, displayed significant negative GCA for GLS resistance. However, in general there was no significant correlation between inbred GCA effects and their performance per se. Only six hybrids combined significant negative SCA for GLS with positive SCA for grain yield, namely A2/A7, A2/A8, K64R/A8, A1/A9, A15/B21 and A3/A7 (Appendix 1).

Table 3.6: GCA effect estimates of parent inbred lines for GLS and grain yield

| Inbred | Gray Leaf Spot | | | | Grain Yield | |
| | Score (1 to 9) | | %Leaf Area Infected | | t/ha | |
	Female	Male	Female	Male	Female	Male
CML442	2.9**	0.3	31.6 **	2.4	-0.4	0.5
CML312	1.3**	1.1*	10.7 *	6.6	-1.5 **	-0.3
CML445	0.8	-0.4	9.9 *	-3.4	-0.1	-0.4
CML395	0.8*	0.4	7.0	4.9	0.0	0.7
CML444	0.2	-0.2	2.8	0.7	0.2	0.2
CML488	0.4	0.3	0.1	-0.9	-0.1	1.4 **
A7	-0.7	2.6**	-8.1	27.4**	0.4	-0.7 *
A8	-0.6	1.3**	-3.4	10.7*	-0.4	-0.6
A9	-1.4**	1.1*	-12.1 *	14.1 *	0.9 *	-0.6
K64R	0.7	1.3*	11.1 *	5.4	-1.3 **	-0.8 *
B11	0.0	---	-4.2	---	-1.3 **	---
B12	-0.8	---	-15.3 **	---	-0.0	---
A13	-2.1 **	-2.2**	-15.6**	-20.1 **	0.7 *	0.5
A14	0.6	2.1**	6.6	23.2 **	-0.1	0.2
A15	-0.9*	-2. 6**	-8.4	-26.8 **	-0.2	0.1
B16	1.6**	0.1	12.4 *	-4.7	0.7 *	-0.4
B17	1.1**	1.3**	13.2 *	16.5 **	0.2	-0.3
B18	-1.7**	-2.7**	-20.1 **	-26.7 **	-1.0 **	-0.5
B19	-1.4**	-1.4**	-13.4 *	-13.9 *	0.7 *	0.3
B20	0.4	-0.4	6.6	-1.7	0.8 *	-0.0
B21	1.4**	-0.6	11.6 *	-1.7	0.8 *	0.1
B22	0.1	-0.8	-0.1	-19.0 **	-0.9 *	0.4
B23	0.3	0.8	0.6	6.6	-0.4	-0.8 *
B24	-1.7**	-0.8	-15.4 *	-9.7	0.1	-1.5 **
CML489	-	-0.4	---	-1.7	---	0.3
A26	-	1.8**	---	13.2 *	---	1.3 **
CIM24	-	-1.7**	---	-12.6 *	---	-0.0
SED	0.57	0.60	6.74	7.16	0.49	0.52
LSD (5%)	1.13	1.21	13.45	14.30	0.98	1.03

**, * = GCA effect significantly different from zero at 1% and 5%, respectively;

--- = No data because line was not used as either male or female.

3.3.6 Heritability Estimates

The heritability estimates for GLS ranged between 49 and 79%, depending on the time and rating method (Table 3.7). Estimates of heritability were significantly higher for the second than the first assessment. Grain texture scores, days to 50% flowering and plant height displayed relatively high heritability estimates (70 to 86%). The regression of hybrid mean on mid parent value were significant (P≤0.05) with coefficients of 1.48 (R^2 = 90.3%) for GLS scores and 0.45 (R^2 = 95%) for grain yield.

Table 3.7: Heritability Estimates for GLS assessment and Selected Agronomic Traits

Character	Environment			Across[*]	
	CED2005	RA2005	ART2005	Average[*]	Adjusted
GLS Rating					
GLS 1 (Score)	56.3	----	--	56.3	---
GLS 2 (Score)	76.2	87.1	74.7	79.3	72
%LAD1	57.5	--	--	49.9	---
%LAD2	68.6	--	--	65.9	---
Agronomic Traits					
Grain Yield (t/ha)	44.3	43.4	45.7	46.8	58.0
Ear Aspect (Score)	41.1	--	--	41.1	---
Plant Aspect (Score)	---	49.1	--	49.1	----
Ears /Plant (No.)	35.2	30.8	--	45.0	40.6
Grain Moisture (%)	81.9	36.4	65.1	60.8	63.9
Grain Texture (Score)	82.4	-----	-----	76.9	86.7
Days to Mid Pollination	---	50.9	75.5	63.2	70.8
Days to Mid Silking	---	55.1	62.4	58.7	70.0
Plant Height (cm)	--	72.6	---	72.6	

[*] Average = arithmetic mean for h^2 across the four environments, which is not adjusted; [*]Across refers to h^2 adjusted for environment and replication effects

3.3.7 Effects of Parents' Resistance on Hybrids and Heterosis

The top 12 resistant hybrids had one or both parents resistant to GLS (Appendix 3). In most instances, the susceptible or the bottom 12 hybrids had both parents as susceptible to GLS. Only two hybrids among the most susceptible (i.e., bottom 12) were crosses between resistant and susceptible (i.e., R x S and S x R) inbred lines. These two hybrids had one common resistant parent (B23), as male in one cross (K64R x B23), and as female (B23 X B17) in the other cross (Appendix 3). The hybrids made between resistant inbred lines (i.e., R x R) exhibited high levels of resistance, which was similar to resistant checks (Table 3.8). Crosses between susceptible (i.e., S x S) inbreds exhibited the lowest levels of resistance (Table 3.8).

The hybrids also showed a large variation for mid-parent heterosis ranging from – 49 to 194.7% for GLS. Although heterosis mean value was positive, eight of the top 12 resistant hybrids, which were formed between resistant and susceptible (R x S and S x R) inbreds had negative heterosis for GLS scores (Appendix 3). Three of five hybrids formed between resistant (R x R) inbreds had positive heterosis and only one hybrid between resistant and susceptible (S x R) displayed positive heterosis for GLS. The most resistant hybrids (top 12) exhibited positive heterosis for grain yield (Appendix 3). Although all hybrids between the different hybrid combinations showed positive heterosis, crosses involving resistant and susceptible inbreds (i.e., R x S and S x R) had the least positive values for GLS (Table 3.8). There were no significant differences for mean yield among the different hybrid categories, but crosses between resistant (R x R) inbreds exhibited highest positive heterosis for grain yield (Table 3.8).

Table 3.8: Resistance and heterosis of hybrids between lines with different resistance level

Category [+]	GLS			Grain Yield		
	Hybrid (Score)	MP (Score)	Heterosis (%)	Hybrid (t/ha)	MP (t/ha)	Heterosis (%)
Inbred Combination						
R X R	2.9	1.7	75.4	5.9	1.9	228.7
R X S	4.0	2.9	40.3	5.9	2.2	178.0
S X R	3.4	2.8	22.5	5.8	2.1	193.9
S X S	6.0	3.7	67.5	6.2	2.5	147.5
Mean of Standards						
Resistant Checks	2.5			5.7		
Susceptible Checks	8.3			5.0		
Mean	4.79			5.91		
SED	0.70			0.58		
LSD (5%)	1.97			1.63		

[+]R = Resistant, S = susceptible; MP = mid-parent heterosis.

3.4   Discussion

3.4.1 Resistance of Inbred Lines and Hybrids

The study indicated existence of high variability for resistance and yield in this germplasm comprising "key" inbreds, their crosses and 24 elite standard hybrids. Breeders can effectively employ the genes from this germplasm or recycle the germplasm to enhance resistance and yield in regionally adapted cultivars. Effectively, it is apparent that breeders do not have to look beyond the region in search of resistant germplasm that lack adaptation to the subcontinent. As early as 1997, Pixley (1997) had observed that ample resistance to GLS could be found from CIMMYT and regional programmes. Gevers *et al*. (1998) reported resistant materials in nurseries at Cedara

during the 1991/2 season. The best breeding approach would involve recycling the adapted regional germplasm, which contain confirmed resistance in both inbred and hybrid form. In addition, most old standard hybrids (SC701, ZS206, R201 and R215) that were bred before the era of GLS (i.e., pre-1988) were classified as susceptible. The newer standard hybrids (SC633, SC635, SC513 and SC627) fitted into either the moderate or resistant category. It is thus suggested that breeders have made some significant progress in breeding for resistance since 1988 when the disease was first recorded in the province of Natal. The challenge that remains therefore would be to enhance yield in the very resistant hybrids, which in most instances had lower relative yield despite the impressive resistance. The following hybrids, which displayed high yield potential and high resistance to GLS would be recommended for release: A9/A15 (relative yield = 109% of overall mean yield), A13/B19 (117%), A7/A15 (113%), A15/B21 (116%), B24/B18 (117%), A9/A13 (119%) and B19/CML444 (111%). These single crosses would also be recommended as sources for new lines in pedigree breeding. In the same vein, the inbred lines B18, B19, A15, A13 and CIM24 which displayed high level of resistance and high yield potential (relative yield ≥100%), and contributed high resistance in their hybrids would be recommended as donors of resistance in breeding programmes.

3.4.2 Environmental Effects

The study revealed significantly large environment, environment x male GCA and environment x SCA interaction for yield and GLS resistance. These were most unlikely to cause difficulty in breeding, since there were no changes in hybrid ranking for resistance, which was supported by the highly positive and significant rank correlations between environments. Environmental differences were explained by differences in levels of disease pressure among the four environments. Hybrids showed differences in magnitude of GLS scores across these environments. The gap between the resistant and the susceptible hybrids was least at ART and significantly wider at Cedara in 2004/5. Unlike at ART and RARS, there were no hybrids that were rated as immune (GLS score = 1.0) at Cedara in 2004/5. Generally, the lack of differences in hybrid ranks between the environments indicated the absence of crossover interaction. Crossover interaction results in change of hybrid ranking hence different sets of selected and rejected hybrids would be obtained in each environment, which complicates selection and impedes breeding progress. Results from this study are in agreement with Lipps *et al*. (1998) who reported that hybrid ranks were similar at 11 locations in the USA over two years despite differences in GLS severity. Consistent ranking of hybrids suggested that hybrids could be evaluated at one reliable site to reduce costs, especially for

national programmes with limited resources. Cedara, which provided the highest discrimination for hybrids and was more reliable with adequate disease pressure in both 2003/4 and 2004/5 seasons, was confirmed as a "hot spot" for disease screening. Locations like RARS and ART were not reliable for screening germplasm for resistance, given that there was even nil GLS pressure during 2003/4. This would impede breeding progress, as breeders would not effect selection for resistance in some seasons. Needless to say that effective breeding for resistance would require reliable disease pressure, which is high enough to discriminate between cultivars every season. It can be argued that a regional disease nursery can be established at Cedara for the benefit of national programmes with limited resources to conduct multilocation tests even in unreliable sites.

3.4.3 Gene Action

Significant mean squares for both GCA and SCA effects indicated that both additive and non-additive gene action, respectively, played a role in conditioning resistance and grain yield in this germplasm. Significantly higher total GCA (86%) than SCA (14%) sums of squares, for the crosses indicated that additive effects had a greater role than the non-additive in conferring resistance. Although the sum of squares for SCA effects were much higher for grain yield (26%) than for GLS scores, GCA accounted for 74% of the variation among hybrids for grain yield. This predominance of additive effects in determining grain yield and resistance suggested that there would be no complications in breeding for resistance and yield, because both GLS-resistance and yield can be improved through selection. Resistance, thus, could be transferred from the identified sources via backcross or recurrent selection procedures that exploit the additive variation. Notably, GCA mean squares due to male sources were at least 1.5 times higher than their female counterparts for resistance, whereas female GCA mean squares were 2.8 times larger than male GCA for grain yield. Since the model used in this study did not include testing for reciprocal effects, it can only be speculated that cytoplasmic genes or maternal effects also played some role in controlling inheritance, especially for grain yield. This suggested that a critical choice should be made about which line would be used as male or female in crosses aimed at enhancing hybrid yield and resistance. If present maternal or cytoplasmic effects could have inflated the GCA mean squares at the expense of SCA. This has serious implications for the interpretation of these results and perhaps for several other studies that overwhelmingly concluded that additive gene action was predominant over the non-additive effects for GLS-resistance. For example, Menkir and Ayodele (2005) reported that GCA accounted for

>70%, while Elwinger *et al* (1990) found that GCA was 1.5 to 11.5 times larger than SCA, yet these and most other studies did not investigate the role of reciprocal effects in conditioning resistance.

Inbreds A13, A15, B18 and B19 consistently showed very high negative GCA effects and significantly conferred their resistance in crosses. These inbreds may be used as resistance donors to enhance resistance in elite, but GLS-susceptible hybrids and synthetic populations. These inbreds had negative GCA both as male and female suggesting that breeders can use them either as male or female depending on other factors. These lines had zero or non-significant GCA for grain yield suggesting that when making single crosses breeders should cross these lines with high yielding lines. Apparently, the inbred line A9, which was susceptible, showed negative GCA as female for GLS and positive as male for grain yield. It is an adapted line that may be conferring "tolerance" type resistance and high yield in crosses. Above all crosses A1 x A9, B18/CML442, A15 x B21 and A7/A15, which combined above average yield, positive SCA effects for yield and negative for GLS should be seriously considered as resistance sources in pedigree breeding.

Negative mid-parent heterosis for GLS-resistance exceeding 10% in crosses involving resistant and susceptible (R X S and S X R), suggested that one resistant parent provided high resistance in single cross hybrids. Thus these hybrids had lower GLS resistance scores, which were lower than the mid-parent; hence these hybrids had higher resistance than the mid-parent. The inbreds A13, A15, B18 and B19 were involved in crosses that displayed negative heterosis for GLS-resistance. These lines could be directly employed as donors of resistance, especially in hybrid oriented programmes. Since non-additive effects were also significant, both dominance and additive gene action could be used to explain the negative heterosis for GLS resistance in crosses between resistant and susceptible inbred lines. These results corroborate those of Cromley *et al*. (2002) who found that single cross hybrids would have adequate level of resistance if at least one parent has resistance. In the current study, resistant lines (A13, A15, B18 and B19) were also associated with crosses that exhibited positive heterosis for grain yield, thus effectively suggesting that they are viable candidates for use in hybrids. Furthermore, their crosses had high relative yield ($\geq$110%) and were better than most standard hybrid checks under the heaviest GLS epidemic at Cedara in 2004/5. Thus, these lines and their hybrids would be recommended for use in breeding programmes as sources of resistance. An analysis of groups of crosses or inbred combinations indicated that R X S (Resistant x Susceptible) had more negative heterosis

than R X R crosses despite displaying similar levels of resistance. Clearly the resistant (R) lines were capable of transmitting their resistance to the single cross hybrid in combination with a susceptible line, suggesting that in addition to additive, dominance action played a role in conditioning resistance. Results implied that these lines could be used as donors to develop highly resistant hybrids. Breeders would select the other line for use in the cross solely on the basis of other important traits. Similarly, it may be suggested that highly performing synthetic populations would be developed by crossing sets of resistant lines to other sets that are selected for yield or other important traits.

3.4.4 Heritability Estimates

Large coefficient values for the mid-parent offspring regression for both GLS and grain yield, indicated that performance of inbreds in hybrids was highly predictable. This was supported by high heritability values for GLS and other important agronomic traits such as ear aspect, grain texture and days to 50% flowering. In the current study, high heritability estimates for GLS were also a reflection of high levels of GCA mean squares. These results are consistent with previous studies, which reported high predictability including high regression values for germplasm that is adapted to mid altitude environments in tropical Africa (Menkir and Ayodele, 2005). Vivek *et al*. (2001) also reported high heritability of 61% for GLS in regional germplasm from east and Southern Africa.   Prediction of high single cross performance using mid-parent offspring regression (R^2 Value between 0.55 and 0.90) has also been reported in temperate maize (Elwinger *et al*., 1990). High heritability estimates of 73 to 78% have also been reported in temperate germplasm (Clements *et al*., 2000; Cromley *et al*., 2002).

3.5 Conclusion

This study was conducted to: (a) evaluate resistance, (b) determine heritability, (c) assess combining ability of inbred lines, and (d) investigate the mode of gene action conditioning resistance to GLS and grain yield in Southern African base germplasm. Results revealed a large proportion (17%) of experimental and new standard hybrids with high levels of resistance to GLS. In addition, the study identified hybrids that combined high resistance to GLS and high yield potential. The following hybrids, which displayed high yield potential and high resistance to GLS would be recommended for release: A9/A15 (relative yield = 109% of overall mean yield), A13/B19 (117%), A7/A15 (113%), A15/B21 (116%), B24/B18 (117%), A9/A13 (119%) and B19/CML444 (111%). Apart from being released, these single crosses could be used as breeding sources for resistant inbred lines in pedigree breeding. The inbred lines A13, A15, B18 and B19, which contributed exceptional levels of resistance to their hybrids, would be

recommended for use as breeding sources. Resistance was highly heritable (72%) and predictable (regression $R^2 = 0.90$). Both additive and non-additive gene action were important in conditioning resistance and grain yield. General combining ability (GCA) effects accounted for 74% and 86% of cross sum of squares grain yield and GLS, respectively, indicating that additive effects predominantly controlled resistance. Hybrids between resistant and susceptible inbred lines showed high levels of resistance, suggesting that single cross hybrids would display adequate resistance when at least one inbred line carries high resistance. A different criterion would be used to select the other line. Large differences between male and female GCA mean squares provided speculation for the role of maternal effects in influencing resistance. Future studies should employ models that incorporate reciprocal effects, to investigate this "speculative" role of maternal effects. It can also be concluded that high resistance to GLS was not associated with low grain yield potential in this set of germplasm.

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Appendices

Appendix 1. Sets of crosses formed in a North Carolina II Mating design (Principal Selection criteria: D = drought tolerance, C = conventional, G = GLS resistance); Conventional means lines were selected on the basis of high yield potential only.

Set 1: Grey Leaf Spot X Drought Tolerant Crosses

♀/♂	CML442 (D)	CML312 (D)	CML445 (D)
B16 (G)			
B17 (G)			
B18 (G)			

Set 2: Conventional X Drought Tolerant Crosses

♀/♂	CML395 (D)	CML444 (D)	CML488 (D)
B19 (C)			
B20 (C)			
B21 (C)			

Set 3: Drought Tolerant X Conventional Crosses

♀/♂	A7 (C)	A8 (C)	A9 (C)
CML442 (D)			
CML312 (D)			
CML445 (D)			

Set 4: Drought Tolerant X Conventional Crosses

♀/♂	CML489 (C)	A26 (C)	CIM24 (D)
CML395 (D)			
CML444 (D)			
CML488 (D)			

Set 5: Conventional X Grey Leaf Spot Resistant Crosses

♀/♂	A13 (G)	A14 (G)	A15 (G)
A7 (C)			
A8 (C)			
A9 (C)			

Set 6: Conventional X Grey Leaf Spot Tolerant Crosses

♀/♂	B16 (G)	B17 (G)	B18 (G)
B22 (C)			
B23 (C)			
B24 (C)			

Set 7: Grey Leaf Spot X Conventional Crosses

♀/♂	B19(C)	B20(C)	B21(C)
A13 (G)			
A14 (G)			
A15 (G)			

Set 8: Conventional X Conventional Crosses

♀/♂	B22(C)	B23(C)	B24(C)
K64R (C)			
B11 (C)			
B12 (C)			

Appendix 2: Hybrids with significant SCA effects for GLS scores and Grain Yield

Female	Male	Gray Leaf Spot		Grain Yield	
		Score (1 to 9)		(t/ha)	
		SCA	Mean Score	SCA	Mean Yield
3	9	-2.6	4.0	0.4	5.8
2	7	-2.1	6.5	0.7*	4.5
1	8	-1.9	7.0	0.6	5.6
1	7	-1.8	8.5	0.1	4.9
2	8	-1.8	5.5	1.1**	4.9
10	24	-1.6	3.0	2.0**	5.2
20	6	-1.4	4.0	-0.1	8.2
18	1	-1.3	2.0	0.2	5.8
3	8	-1.3	5.5	0.4	5.7
1	9	-1.3	7.5	1.3**	6.3
15	21	-1.3	2.0	1.0*	6.9
12	23	-1.2	3.5	0.3	5.4
19	5	-1.1	2.0	-0.3	6.6
3	7	-1.1	7.0	1.3**	6.4
2	9	-1.1	6.0	0.3	4.2
5	25	-1.0	3.5	0.0	6.6
16	2	-0.9	6.5	-0.1	6.4
16	3	-0.9	5.0	0.2	6.6
4	26	-0.8	6.5	0.2	7.6
19	4	-0.8	3.0	-1.2**	6.3
4	25	-0.7	4.5	-0.2	6.2
5	27	-0.7	2.5	-0.6	5.6
8	14	0.7	7.0	-0.3	5.6
9	15	0.7	1.5	0.7*	6.4
13	20	0.7	3.0	0.3	7.0
12	22	0.8	4.0	0.0	6.5
16	1	0.9	7.5	0.0	7.3
20	4	0.9	6.5	-0.8*	6.8
9	13	0.9	2.0	-0.4	7.1
7	14	0.9	7.0	-0.8*	5.9
9	14	1.1	6.5	0.2	7.4
22	16	1.1	6.0	1.6*	6.2
15	20	1.1	4.5	-1.0*	4.8
7	13	1.2	3.0	-0.2	6.8
23	17	1.2	7.5	1.0*	6.3
19	6	1.4	5.0	-0.8*	7.3
8	15	1.4	3.0	-0.3	5.4
13	21	1.4	3.5	-0.5	6.3
24	18	1.7	2.0	1.3**	6.9
10	23	1.8	8.0	0.5	4.4
14	21	2.2	7.0	-0.8*	5.2
15	19	2.6	5.0	-0.3	5.9
SED		0.51	0.98	0.43	0.84
LSD $_{0.05}$		1.01	1.95	0.86	1.67

All values for GLS are significant; but for grain yield **, * = SCA effects significant at 1% and 5%, respectively.

Appendix 3: Resistance and Heterosis of Selected hybrids for GLS and Grain Yield

Entry	Female Inbred	Male Inbred	Inbred Combination	GLS Score (1 to 9)			Grain Yield		
				Hybrid	MP	Heterosis	Hybrid	MP	Heterosis
Top 12: Resistant Hybrids:						(%)	(t/ha)	(t/ha)	(%)
35	13	19	RXR	1.5	1.9	-19.8	6.9	2.3	197.5
28	9	15	SXR	1.5	2.9	-49.1	6.4	2.4	172.1
54	19	5	RXS	2.0	3.0	-34.3	6.6	2.7	140.6
50	18	1	RXS	2.0	2.5	-20.2	5.8	2.4	134.9
43	15	21	RXR	2.0	2.3	-14.1	6.9	2.3	202.1
64	22	18	RXR	2.0	1.5	34.4	4.4	1.8	138.1
67	23	18	RXR	2.0	1.8	10.2	5.2	2.0	157.4
70	24	18	RXR	2.0	1.5	34.4	6.9	1.7	312.5
22	7	15	SXR	2.0	3.0	-33.3	6.7	2.6	157.9
26	9	13	SXR	2.0	2.9	-31.9	7.1	2.3	212.8
23	8	13	SXR	2.5	2.0	22.8	5.7	2.5	129.7
15	5	27	SXR	2.5	2.7	-7.9	5.6	2.7	104.3
Bottom 12: Susceptible Hybrids:									
4	2	7	SXS	6.5	4.9	32.3	4.4	2.7	62.0
11	4	26	SXS	6.5	4.5	44.4	7.6	2.6	191.3
14	5	26	SXS	6.5	4.4	48.5	8.4	2.4	251.3
45	16	2	SXS	6.5	5.0	30.2	6.4	2.2	190.9
24	8	14	SXS	7.0	2.9	141.3	5.6	2.5	119.0
40	14	21	SXS	7.0	3.2	119.8	5.2	2.2	130.1
7	3	7	SXS	7.0	3.7	88.7	6.4	2.8	128.4
2	1	8	SXS	7.0	2.8	152.1	5.6	2.6	114.0
21	7	14	SXS	7.0	3.9	81.4	5.9	2.6	131.2
48	17	2	SXS	7.0	5.0	38.7	5.7	2.6	118.1
66	23	17	RXS	7.5	3.2	135.9	6.3	2.1	201.6
44	16	1	SXS	7.5	3.8	96.6	7.3	2.1	243.8
3	1	9	SXS	7.5	3.7	103.9	6.3	2.4	164.2
29	10	23	SXR	8.0	2.8	181.1	4.4	1.5	184.9
1	1	7	SXS	8.5	3.7	127.6	4.9	2.6	87.7
Standard Check Hybrids:				2.5					
SC627			R	1.8			5.4		
ZS257			R	2.4			5.0		
SC513			R	2.4			5.4		
SC709			R	2.5			5.2		
Susceptible Checks				8.3					
SR52			S	6.9			6.5		
R201			S	8.4			4.4		
R215			S	8.7			4.4		
SC701			S	8.8			4.6		
ZS206			S	8.9			5.3		
Overall Mean				4.79			5.91		
SED				0.70			0.58		
LSD (%)				1.97			1.63		

R = resistant, S = susceptible to GLS; MP = mid-parent value.

Chapter 4: Phaeosphaeria Leaf Spot Resistance in Southern African Maize base Germplasm

Abstract

Phaeosphaeria leaf spot (PLS) is a relatively recent disease of maize in Southern Africa, but with potential to become a major disease thereby posing a threat to food security. Very little is known about the level of resistance and the mode of inheritance in regional materials. This study was therefore conducted to determine: (a) levels of resistance; (b) heritability; and (c) gene action conditioning resistance in Southern African maize. Twenty-seven inbred lines were mated in eight sets according to a North Carolina Design II scheme resulting in 72 hybrids. Inbred lines, experimental hybrids and 24 standard hybrids were evaluated in two replications and three environments at Cedara in South Africa and Rattray Arnold Research Station in Zimbabwe, during 2003/4 and 2004/5 seasons. Disease scores could be divided into seven classes. Resistance was not only expressed by reduced severity but also by reduced lesion number and size. Thirty-four percent (34%) of the hybrids and 37% of the inbred lines were resistant. Resistance was highly heritable in the narrow sense (62% to 73%). Late disease observations appeared to give higher heritability scores than earlier disease observations just after flowering and assessments of leaf area diseased appeared to give higher heritability scores than disease scores. General combining ability (GCA) effects (90%) were highly significant (P<0.01), while specific combining ability (SCA) effects (10%) were not significant, indicating that mainly additive gene action played a role in conditioning resistance. It is thus suggested, that in breeding resistant hybrids, both parents should carry high levels of resistance. However, female GCA effects contributed 58% and male GCA effects accounted for 32%, suggesting that future models should include reciprocal effects to investigate the possible role of maternal effects in influencing resistance. The following experimental hybrids, which combined high levels of resistance with high relative yield (121 to 141% of overall mean yield) are recommended for further testing and subsequent release: CML444/A26 (141.9%), B20/CML488 (138%), B19/CML488 (124%) and B21/CML395 (121%). Inbred lines B23, B17, B12 and CML444, which showed significant contribution to resistance, would be recommended for use as breeding sources. The study showed that hybrids which combine high levels of resistance to PLS and high yield potential could be developed.

4.1   Introduction

Phaeosphaeria leaf spot (PLS) caused by the fungus *Phaeosphaeria maydis* (Henn) Rane, Payka and Renfro appeared recently in sub-Saharan countries, namely South Africa, Zimbabwe, Kenya and Cameroon (Carson, 1999). The disease has been reported to be prevalent in high rainfall, moderate temperature and high altitude areas in Brazil, Asia and Africa (Carson *et al*., 1991; Carson, 2001; Silva, 2004). Although Flett (2004) reported that PLS occurred after grain filling and that grain yield had no significant correlation with the disease in South Africa, Pegoraro *et al*. (2001) reported a significant correlation (r = 0.45) between grain yield and PLS severity in Brazil. Paccola-Meirelles (2001) reported a yield reduction of 63% in susceptible cultivars in Brazil. According to Paccola-Meirelles (2001), PLS causes yield losses through accelerated leaf senescence, reduced plant cycle and decreased grain size and weight. Although not reported in high epidemic proportions, incidence of PLS has been increasingly observed in the Southern African region for the past five years (Vivek *et al*., 2001; Carson, 2005), hence the disease has the potential to pose a serious threat to regional food security.

Carson (2001, 1999) studied resistance to PLS in two major heterotic groups used in the USA hybrid programmes and reported that inbreds of the B73-type were more susceptible than those related to Mo17. Studies of the mode of inheritance have reported predominance of additive effects and a smaller role of dominance action in conferring resistance in American and Brazilian materials (Silva and Moro, 2004; Carson, 2001; Carson *et al*., 2000). Silva and Moro (2004) reported that SCA effects and their interaction with environments were not significant. Four to six quantitative trait loci (QTLs) have been reported to be involved in controlling resistance in Mo17 (Carson *et al*., 2005; Carson, 2000; Carson *et al*., 1996). Carson *et al*. (2005) also reported that additive x additive interaction was also significant in QTL studies. Carson (2001) reported involvement of incomplete dominance, controlled by three to four genes in Mo17, whereas Pegoraro *et al*. (2002) reported two major independent genes that acted in an additive manner in conferring resistance in Brazilian maize.

Carson (2001) reported that heritability of resistance was high (70 to 85%) and there were low levels of cultivar x environment interaction effects. Most studies have been conducted using USA and Brazilian material. In Southern Africa, the area grown to USA and Brazilian germplasm is not of any significance. Furthermore, the management and environmental conditions in the Americas are likely to be different from those in Southern Africa. Published literature about resistance and the mode of inheritance in regional

materials is scarce. Resistance of local germplasm to PLS should, therefore, be evaluated to obtain knowledge and make appropriate strategies to accumulate or enhance resistance before the damage caused by PLS reaches economic levels. This study was conducted to determine: (a) levels of resistance; (b) heritability; and (c) gene action conditioning resistance in a selected set of Southern African maize.

4.2   Materials and Methods

4.2.1 Germplasm

Inbred lines used in the study comprised a sample from the following heterotic groups and their derivatives: a) CIMMYT (International Maize and Wheat Improvement Centre): A and B; and b) Southern African: P, K64R, SC, N3, M and I group (Table 4.1). Gevers and Whythe (1987) and Mickelson *et al*. (2001) presented detailed descriptions of these groups. The 27 inbred lines used consisted of six GLS-resistant and six drought-tolerant ones from CIMMYT, and 15 conventional lines obtained from Seed Co in Zimbabwe. These inbred lines are adapted to tropical east and Southern Africa. Inbred lines were divided into eight sets of three each; hence three inbred lines in one set were used as females and crossed with three inbred lines from another set used as males (Appendix 1), according to a North Carolina Design II Mating Scheme (Comstock and Robinson, 1948; 1952; Hallauer and Miranda, 1988). Each inbred line was used once as a female parent in one set and once as a male parent in another set, except the inbreds K64R, B11 and B12, which were used as females only, and CML489, A26 and CIM24, which were used as male parents only (Appendix 1, in sets 8 and 4, respectively). The inbreds CML489, CIM24 and A26 replaced K64R, B11 and B12 as male in set 4, which had failed to provide adequate pollen due to severe attack by maize streak virus. Consequently, eight sets of hybrids comprising nine hybrids each were formed among the 27 inbreds. The 27 inbred lines, their 72 crosses and 24 standard hybrids that were used as controls effectively constituted the reference population for this study. Among the 24 standard hybrids SC627 and SC513 were used as GLS resistant check hybrids, while SC403, SC633, PAN6777, ZS255, ZS257, R201 and R215 were widely grown hybrids throughout Southern Africa. The PLS resistance of the standard hybrids was not known at the beginning of the study.

Table 4.1: Parent Inbred Lines used in a Design II Mating Scheme

Inbred	Designation	Heterotic Group	Principal Selection Criteria
1	CML442	A	Drought Tolerance
2	CML312	A	Drought Tolerance
3	CML445	AB	Drought Tolerance
4	CML395	B	Drought Tolerance
5	CML444	B	Drought Tolerance
6	CML488	B	Drought Tolerance
7	A7	M	Conventional
8	A8	M	Conventional
9	A9	P	Conventional
10	K64R	K	Conventional
11	B11	K	Conventional
12	B12	K	Conventional
13	A13	A	GLS Resistance
14	A14	A	GLS Resistance
15	A15	N	GLS Resistance
16	B16	I	GLS Resistance
17	B17	B	GLS Resistance
18	B18	B	GLS Resistance
19	B19	K	Conventional
20	B20	KB	Conventional
21	B21	K	Conventional
22	B22	S	Conventional
23	B23	S	Conventional
24	B24	S	Conventional
25	CML489	AB	Conventional
26	A26	I	Conventional
27	CIM24	A	Drought Tolerance

4.2.2 Experimental Design

Hybrids were evaluated at Cedara (CED; 1076 m altitude) in South Africa and Rattray Arnold Research Station (RARS; 1350 m altitude) in Zimbabwe. Due to inadequate seed for some crosses, only 64 hybrids comprising 57 experimental and seven standard hybrids were evaluated in 2003/4 and the experiments were laid out as 8 X 8 simple lattice design. Ninety-six hybrids comprising 72 experimental and 24 hybrid checks were screened in 2004/5 and the experiments were laid as 12 X 8 alpha lattice block designs with two replications. Inbred parent lines were evaluated in 6 X 5 α-lattice designs at RARS in 2004/5. Established plant populations (plants/ha) were 44000 at Cedara and 53000 at RARS, while fertiliser (kg/ha) was applied as follows: 120 N: 33 P: 44 K at Cedara and 208 N: 35 P: 21 K at RARS. Total precipitation was as follows: 853 mm at Cedara (2003/4); 826 mm at RARS (2004/5) and 885 mm at Cedara (2004/5). Standard cultural practices, including hand planting, hand weeding and application of herbicides was followed, and fields were left to natural disease inoculation. Disease development was monitored every fortnight beginning from tassel emergence. *Phaeosphaeria* leaf spot severity was assessed at 50% silk emergence (PLS1) and at hard dough stages (PLS2) based on the visual assessment of the whole plot by estimating (i) percentage

leaf area diseased or necrotic (% LAD) and (ii) a rating scale of 1 to 9. Using this scale, 1 = no disease, 3 = lesions on lower leaves, 5 = disease on most leaves and lower leaves dead, 7 = lower leaves dead and many lesions on all leaves above the ear and 9 = almost all leaf tissue is dead or necrotic (Munkvold *et al.*, 2001). At harvest grain yield was measured on a whole plot basis following standard practice used at CIMMYT (CIMMYT, 1985) and was adjusted to 12.5% moisture using the formula:

Grain Yield (t/ha) = [Grain Weight (kg/plot) x 10 x (100-MC)/ (100-12.5)/(Plot Area)], where MC = Grain Moisture Content.

Mid parent heterosis (MPH) for PLS was calculated for each hybrid (Falconer, 1989) using the formula:

$$MPH (\%) = (F_1-MP)/MP \times 100,$$

Where F_1 = mean of the F_1 hybrid performance, MP = mean of two parents making the cross using the formula: $(P_1 + P_2)/2$, where P_1 and P_2 are the means of the inbred parents.

4.2.3 Statistical Analyses

General analyses of variance were performed for all hybrids and inbred lines, including control hybrids using IRRISTAT (2003) computer package for PLS and grain yield data for each site. Genetic analyses for PLS were performed in SAS (SAS Institute, 1997) as a fixed effects model for experimental hybrids, as described by Hallauer and Miranda (1988) for across environments using the following linear model:

$Y_{ijkpq} = \mu + S_p + gi(S_p) + g_j(S_p) + h_{ij}(S_p) + E_q + r_k(SE)_{pq} + (ES)_{pq} + (Eg)_{iq}(S_p) + (Eg)_{jq}(S_p) + (Eh)_{ijq}(S_p) + e_{ijkpq}$

Where i =1, 2, 3; j = 1, 2, 3; k = 1, 2; p = 1, 2, 3, 4, 5, 6, 7, 8; q = 1, 2 and Y_{ijkpq} denotes the value of the hybrid of a mating of the ith female line, the jth male line, in the kth block, within set p and in the qth environment. The terms are defined as follows:

μ = Grand mean
S_p =   the average effect of the pth set
$g_i(S_p)$ = the GCA effect common to all hybrid of the ith female line nested within p^{th} set,
$g_j(S_p)$ = the GCA effect common to all hybrid of the jth male line nested within p^{th} set,
$h_{ij}(S_p)$ = the SCA effect specific to hybrid of the ith female and j^{th} male line nested within p^{th} set,
E_q = average effect of qth environment
$r_k(SE)_{pq}$ = the effect of the kth replication nested within the pth set and q^{th} environment

$(ES)_{pq}$ = the interaction between set effects and the environment

$(Eg)_{iq}(S_p)$ and $+(Eg)_{jq}(S_p)$ = the interaction between environment and GCA nested within sets

$(Eh)_{ijq}(S_p)$ = the interaction between environment and SCA nested within sets

e_{ijkpq} = the random experimental error.

According to Hallauer and Miranda (1988), the main effects due to females (sets) and males (sets) are equivalent to the GCA (general combining ability), while male x female (sets) interaction effects represent the SCA (specific combining ability) effects in a diallel mating. General combining ability and SCA effect estimates for inbred lines and crosses, respectively, were determined by line x tester analysis in Agrobase (2005) computer package. Using the variance ratios in REML (GenStat, 2003), heritability estimates were calculated as suggested by Hallauer and Miranda (1988) for the fully inbred parents (F = 1) (where $\sigma^2_m = \sigma^2_f = \frac{1}{2}\sigma^2_A$; and $\sigma^2_{mf} = \sigma^2_D$) using the formulae:

a) $h^2 = 2\sigma^2_m/(\sigma^2/r + \sigma^2_{mf} + 2\sigma^2_m)$ for one environment, and

b) $h^2 = 2\sigma^2_m/(\sigma^2/re + \sigma^2_{fme}/e + 2\sigma^2_{me}/e + \sigma^2_{mf} + 2\sigma^2_m)$ for across environments,

Where σ^2_m = male (set) variance, σ^2 = random error variance; σ^2_{mf} = male x female (set) variance; σ^2_{fme} = environment x male x female (set) variance; σ^2_{me} = environment x male (set) variance; r = number of replications and e = number of environments. σ^2_f = fema (set) variance; σ^2_A = additive variance and σ^2_D = dominance variance. Heritability estimates were calculated using the male variance (σ^2_m) to avoid the upward bias of heritability due to maternal effects if the female variance (σ^2_f) was used.

4.3   Results

4.3.1 Resistance of Inbred Lines

The PLS disease intensified after the grain filling stage at RARS. Consequently, very few inbred lines had PLS rating greater than 3, but the most susceptible inbred CML445 had a rating of 6.1 while four of the most resistant showed no symptoms. There were no inbred lines with a severity rating of 7.0 to 9.0, but there were significant (P<0.05) differences among inbred lines for reaction to PLS (Table 4.2). For convenience of the study inbreds were divided into five classes of resistance as follows: (a) immune (PLS score = 1.0); (b) resistant (1.1 to 1.2); (c) moderate resistance (1.3 to 1.5); (d) moderately susceptible (1.6 to 2.6); and (e) susceptible (2.7 to 6.1). Inbreds in the resistant category had scores that were significantly less than the mean; those in the moderate classes had PLS rating similar to the mean, while the susceptible had ratings

significantly larger than the mean. Resistance was not only expressed by reduced severity but also by reduced lesion number and/or reduced lesion size. Four out of 27 or 15% of the inbred lines were immune to PLS disease: B17, B12, CML444 and A9. The five most susceptible inbreds were: B18, B21, K0315Y and CML445 (Table 4.2).

Table 4.2: PLS resistance score for parent inbred lines used in a design II mating scheme

Entry	Inbred	Category	Disease Score (1 to 9)♣
17	B17	Immune	1.0 *
12	B12	Immune	1.0
5	CML444	Immune	1.0
9	A9	Immune	1.0
22	B22	R	1.1 **
15	A15	R	1.1
6	CML488	R	1.1
10	K64R	R	1.1
4	CML395	R	1.1
7	A7	R	1.1
16	B16	MR	1.3 **
26	CIM24	MR	1.4
23	B23	MR	1.4
2	CML312	MR	1.4
25	CML489	MS	1.7 ***
1	CML442	MS	1.7
27	A26	MS	1.7
24	B24	MS	1.8
20	B20	MS	1.8
19	B19	MS	2.0
13	A13	MS	2.2
14	A14	MS	2.2
11	B11	MS	2.9
18	B18	S	3.1
21	B21	S	3.2
8	A8	S	3.5
3	CML445	S	6.1
	Mean		1.9
	SED		0.3
	LSD $_{0.05}$		0.7

♣ R= resistant, MR = Moderately Resistant and S = Susceptible; * Immune, **Traces of necrotic spots are clearly noticeable; ***Coalescing necrotic spots.

4.3.2 Hybrid Resistance

Severity of PLS was significantly higher in 2004/5 than 2003/4 at Cedara and Rattray Arnold Research Stations. There was a significant (P<0.01), positive correlation between

rating scores and % LAD (r = 0.93) and the rank correlation between the environments was also significant and positive (r = 0.77). Disease scores for hybrids in each environment are presented in Appendix 1. At Cedara, PLS scores ranged from 0.9 to 4.6 (CML445/A8) in 2003/4 and from 1.1 (K64R/B23) to 7.1 for the susceptible control SC513 in 2004/5. At Rattray Arnold, there were moderate levels of PLS ranging from nil to traces between 1.0 and 4.5 (CML445/A8) in 2004 for most experimental hybrids. The susceptible control, SC403 had high infections of 7.0 in 2004/5. Hybrids were categorised into "resistance" classes using data obtained at Cedara during 2004/5, because disease pressure was most severe in that environment (Fig. 1). Disease scores could be classified in seven statistical classes. Resistance was not only expressed by reduced severity but also by reduced lesion number and/or reduced lesion size. Thirty-four percent (34%) of the hybrids were resistant to PLS (Fig. 1). Table 3 shows the resistance scores and grain yield of the hybrids at Cedara during 2004/5. The most resistant hybrids were crosses between resistant inbred lines, while in general the most susceptible were crosses between susceptible inbred lines. The following experimental hybrids combined high level of resistance with high relative grain yield (i.e. 121 to 141.9% of overall mean yield): CML444/A26 (141.9%), B20/CML488 (138%), B19/CML488 (124%) and B21/CML395 (121%) (Table 4.3). Among the susceptible hybrids (bottom 15), there were also hybrids with high relative grain yield: A9/A13 (119%), A7/A13 (115%) and B24/B18 (117%). Although two standard hybrids (R201 and R215) were ranked among the most resistant they had relatively low relative yield (75%). Three standard hybrids SC403, ZS255 and SC513 were among the most susceptible with relatively low yield (≤ 91%). The most resistant hybrids (top three) had one common resistant parent (B23), while the most susceptible contained three common susceptible parents (B24, A8 and CML445).

Generally, hybrids' resistance declined with declining resistance level of the parent-inbred lines (Table 4.4). Hybrids formed between resistant inbreds (R x R), and between resistant and moderately resistant (R x MR) had high level of resistance, while those formed between susceptible lines were most susceptible. The most resistant hybrids (in the R x MR category) were more resistant than the mean of control hybrids and susceptible control hybrids. Although there was no significant relationship between grain yield and PLS resistance, there were hybrids that combined high levels of resistance with high grain yield. In particular crosses between resistant R and moderately susceptible MS inbreds and vice versa appeared to be high yielding.
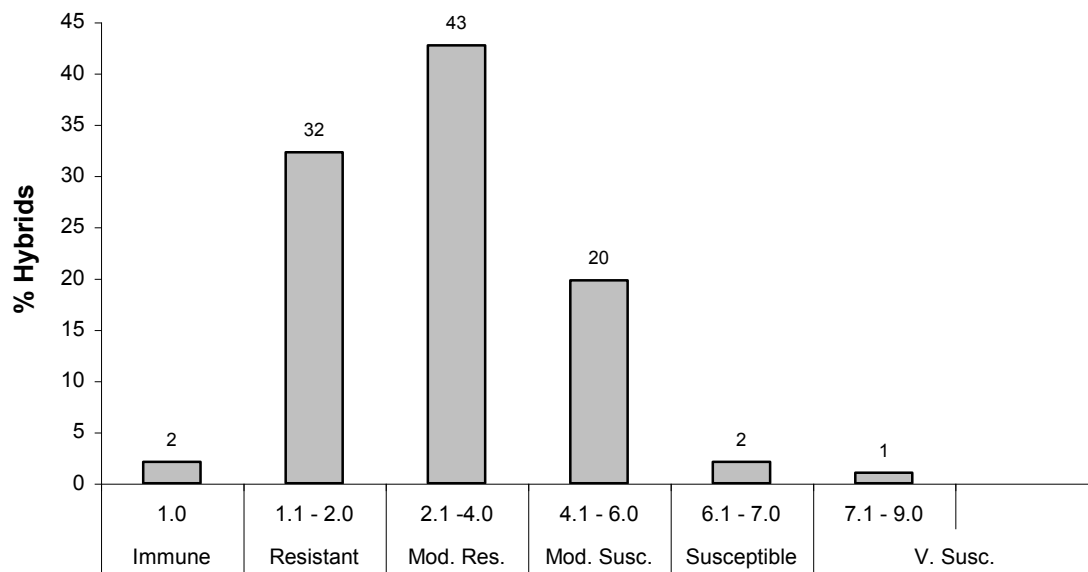
Fig. 1: Frequency distribution of 96 hybrids for resistance to PLS at Cedara in 2004/5.

Table 4.3: Mean PLS Rating and Grain Yield of selected hybrids at Cedara 2004/5

Entry	Name[♠]	Category	PLS		Yield	
			Score	%LAD	t/ha	% Mean
Top 21 Resistant hybrids						
69	B12/B23	R X R	1.0	0.3	5.4	91.2
47	B23/B16	R X MR	1.0	0.2	5.4	91.3
67	K64R/B23	R X R	1.1	0.3	4.4	74.3
49	B22/B17	R X R	1.2	0.3	4.5	76.4
66	B12/B22	R X R	1.3	0.2	6.4	**109.3**
29	CML444/CML489	R X R	1.3	0.3	6.6	**111.6**
36	CML488/CIM24	R X MR	1.4	0.1	6.1	104.2
46	B22/B16	R X MR	1.4	0.3	6.2	104.5
12	B21/CML395	S X R	1.4	0.8	7.1	**121.1**
65	04C2181	S X R	1.4	0.4	5.3	89.1
79	**R 201**	Control	1.5	0.3	4.4	75.3
18	B21/CML488	S X R	1.6	0.4	6.9	**116.4**
32	CML444/A26	R X MS	1.6	0.0	8.4	**141.9**
4	B16/CML312	MR X MR	1.7	1.0	6.4	**107.9**
13	B19/CML444	MS X R	1.8	2.5	6.6	**111.6**
11	B20/CML395	MS X R	1.9	1.0	6.8	**114.6**
80	**R 215**	Control	1.9	0.1	4.4	75.1
17	B20/CML488	MS X R	1.9	1.3	8.2	**138.3**
10	B19/CML395	MS X R	1.9	1.0	6.3	**106.2**
16	B19/CML488	MS X R	2.0	2.6	7.3	**124.4**
2	B17/CML442	R X MS	2.0	1.5	6.7	**113.7**
Bottom 17 susceptible hybrids						
71	B11/B24	S X MS	4.1	21.6	3.8	64.1
22	CML442/A8	MS X S	4.2	16.3	5.5	93.5
44	A8/A15	S X R	4.4	27.5	5.4	91.8
39	A9/A13	R X S	4.4	29.6	7.0	**119.0**
51	B24/B17	MS X R	4.5	10.5	6.2	**105.8**
70	K64R/B24	R X S	4.5	20.0	5.2	88.2
6	B18/CML312	S X MR	4.6	25.5	5.1	86.4
21	CML445/A7	S X R	4.7	25.7	6.4	**107.9**
37	A7/A13	R X S	4.8	25.7	6.8	**115.5**
38	A8/A13	S X S	4.9	35.9	5.7	96.8
74	**SC403**	Control	5.1	19.9	5.0	85.1
3	B18/CML442	S X MS	5.1	25.2	5.8	98.1
24	CML445/A8	S X S	5.1	27.7	5.7	96.1
48	B24/B16	S X MR	5.6	39.7	5.2	88.2
78	**ZS255**	Control	5.9	49.9	5.0	85.4
54	B24/B18	MS X S	6.3	49.9	6.9	**117.5**
73	**SC513**	Control	7.1	44.9	5.4	91.1
Means			2.9	10.8	5.9	100.2
SED			0.6	5.8	0.6	
LSD $_{0.05}$			1.7	16.3	1.6	

[♠] Control hybrids are in bold; and high yield values are in bold

Table 4.4: Resistance and Grain Yield of different Groups of hybrids

Group	Category	PLS		Yield	Relative Yield
		%LAD	Score	(t/ha)	(% Overall Mean)
1	R X MR	0.7	1.6	5.9	99.5
2	R X R	2.5	1.7	5.7	97.0
3	R X MS	3.1	2.1	6.4	108.3
4	MS X R	3.3	2.3	6.4	108.9
5	MR X R	7.1	3.3	4.3	72.1
6	S X R	7.6	2.6	6.1	103.8
7	S X MS	14.5	3.7	5.7	96.2
8	R X S	15.1	3.5	6.0	100.9
9	Control	18.2	3.5	5.2	88.5
10	S X S	24.0	4.0	5.4	91.7
11	MS X S	25.3	4.5	5.9	99.4
12	S X MR	32.6	5.1	5.2	87.3
Mean		10.8	2.9	5.9	100
SED		5.8	0.6	0.6	
LSD $_{0.05}$		16.3	1.7	1.6	

R = resistant, S = susceptible, MS = moderately susceptible, MR = moderately resistant

4.3.3 Gene Action and Combining Ability Effects for Inbred Lines

Environments, sets and the environment x sets interaction mean squares were highly significant (P<0.01) for PLS scores across environments (Table 4.5). Male GCA and female GCA effects were highly significant for PLS scores. In terms of interaction with the environment, only the environment x female GCA interaction effect was significant (P<0.01). In total, GCA effects accounted for 90% of the sum of squares due to crosses with female GCA explaining 58% and males 32%. Although not significant, SCA effects accounted for 10% of the sum of squares for the crosses. The inbreds CML444, CML488, B11, B12 and B22 had negative GCA effects as both male and female parents (Table 4.6). The inbred lines CML445, CML395, B16, B17 and B23 had negative GCA as female, while K64R, A15, CML489, CIM24 and B20 displayed negative GCA as male sources.

Table 4.5. Mean Square Values of Hybrids for *Phaeosphaeria* Leaf Spot across two Environments (RARS and CED) in 2004/5

Source of Variation	D.F.	PLS
Environment	1	110.967**
Set	7	5.748**
Environment x Set	7	4.976**
Replication (Set) (Environment)	16	2.516
Female (Set)	16	5.514**
Male (set)	16	3.014**
Female x Male (Set)	31	0.493
Environment x Female (Set)	16	1.793**
Environment x Male (set)	16	0.575
Environment x Female x Male (Set)	31	0.886

**, * Significant at 1% and 5%, respectively.

Table 4.6. GCA Effect Estimates of Parent Inbred Lines for PLS at three environments

Entry	Inbred	CED2004				CED2005				RA2005	
		%LAD		Score		%LAD		Score			
		Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
1	CML442	1.3	19.6 **	0.5	0.3	0.2	0.9	-0.5	-0.7 *		
2	CML312	6.3 *	13.8 **	0.3	0.0	-0.1	0.7	0.5	-0.2		
3	CML445	-7.0 *	23.0 **	1.3 **	0.0	10.6 **	1.6	2.9 **	1.2**		
4	CML395	-9.5 **	-5.4	-0.8 *	-1.0	-8.9 **	-8.3 **	1.7 *	-0.7 *		
5	CML444	-8.3 *	-7.9 *	-1.3 **	-0.8 *	-11.6 **	-7.6 *	1.9 **	-0.8 *		
6	CML488	-9.5 **	-8.7 *	-0.8 *	-1.0 *	-4.8	-7.6 *	0.6	-0.8 *		
7	A7	20.7 **	4.6	0.8 *	0.5	3.2	0.2	-0.5	0.5		
8	A8	24.6 **	14.6 **	1.3 **	1.2 **	13.7 **	9.9 **	0.0	1.4 *		
9	A9	14.6 **	4.3	0.7	0.5	5.6 *	0.6	-0.7 *	1.0 *		
10	K64R	-5.4	-7.0 *	0.1	-1.2 **	1.3	-8.2 **	0.8 *	3.7 **		
11	B11	-7.9 *	-7.0 *	0.6	---	2.5	---	2.1 **	--		
12	B12	-8.7 **	-9.0 *	-1.6 **	---	-10.1 **	---	0.1	--		
13	A13	10.5 **	0.8	-0.2	1.8**	0.6	21.2 **	-0.7 *	-0.5		
14	A14	-1.5	-4.5	0.1	0.3	0.6	-5.1	0.0	-0.2		
15	A15	14.6 **	-9.3 **	-0.6	0.7	-5.3 *	6.4	-0.7 *	-0.4		
16	B16	-8.3 *	-4.5	-0.6	0.0	-5.6 *	4.6	-0.3	-0.3		
17	B17	-8.3 *	-3.7	-0.5	-0.2	-5.8 *	-4.4	-0.2	-0.3		
18	B18	-4.5	8.8 **	1.5**	1.3**	14.6 **	14.7 **	0.9 *	0.7 *		
19	B19	---	---	-0.6	0.2	-6.6 *	0.6	-0.8 *	-0.6 *		
20	B20	-3.7	-9.5 **	-1.0 *	-0.8 *	-8.4 **	-4.4	-0.8 *	-0.8 *		
21	B21	-4.0	-8.7 **	-1.2 **	0.0	-8.4 **	-0.3	-0.7 *	0.1		
22	B22	-6.2 *	-8.7 **	-0.6	-1.2 **	-2.9	-8.8 **	-0.8 *	-0.8 *		
23	B23	-9.2 **	-5.4	-1.0 *	-1.3 **	-6.9 *	-8.6 **	-0.8 *	0.0		
24	B24	--	---	2.8 **	1.9 **	24.7 **	12.5 **	1.7 *	3.3 **		
25	CML489	---	---	---	-0.8 *	--	-7.4 *	---	-0.7 *		
26	A26	---	---	---	-0.5	--	-2.6	---	-0.4		
27	CIM24	--	--	---	-0.8 *	--	-7.8 *	--	-0.8 *		
SED		4.11	4.12	0.501	0.532	3.728	3.962	0.430	0.457		

**, * = GCA effect significantly different from zero at 1% and 5%, respectively; --- = no data because the line was not used either as male or female; CED2004 and CED2005 = Cedara in 2003/4 and 2004/5, respectively. RA2005 = RARS in 2004/5.

4.3.4 Heritability of Resistance

Heritability of resistance was higher for the second (at hard dough stage) than the first rating (at 50% silk emergence) for both PLS scores and % LAD (Table 4.7). Average heritability was 52% and 67% for the first (PLS1) and second (PLS2) rating scores, respectively. Late disease ratings appeared to give higher heritability scores than earlier disease ratings just after flowering. However, larger heritability estimates were obtained for LAD of 62% for first and 73% for second rating. Overall heritability estimate was 68%. The parent on offspring regression was significant (P<0.01) with a coefficient value of 0.62 for PLS scores.

Table 4.7. Heritability Estimates (%) for hybrids' reaction to PLS at three environments and four methods of assessment

Rating	Ced2005	Ced2004	RA2005	Average
PLS1 Score	51.69	--	--	51.69
PLS2 Score	61.96	--	73.50	67.73
%LAD1	--	62.01	---	62.01
%LAD2	71.76	73.93	--	72.85

Ced2005, Ced2004 = Cedara in 2004/5 and 2003/4, respectively; RA2005 = Rattray Arnold in 2004/5; Average = arithmetic mean of PLS values across the three environments.

4.3.5 Heterosis of Hybrids for Resistance

Heterosis for PLS scores ranged from -53.4 for the hybrid 21 x 4 to 259.7% for hybrid 24 X 6 (Table 4.8). Although average heterosis was 53.89%, 13 hybrids displayed significant negative heterosis for PLS. All hybrids with negative heterosis for PLS scores were in the resistance category, with resistance in the male, the female or both parents.

Table 4.8. Mid Parent (MP) heterosis of selected hybrids for PLS scores at Ced2005

Female	Male	Category	Hybrid	MP	Heterosis (%)
Top 13 Hybrids:					
21	4	S X R	1.0	2.1	-53.4
18	3	S X S	3.0	4.6	-34.8
16	3	MR x S	2.5	3.7	-31.7
21	6	S X R	1.5	2.1	-30.1
3	9	S X R	2.5	3.5	-29.4
17	3	R X S	2.5	3.5	-27.6
23	16	R X MR	1.0	1.3	-24.6
10	23	R X R	1.0	1.3	-20.0
12	23	R X R	1.0	1.2	-14.8
23	18	R x S	2.0	2.3	-11.7
15	21	R X S	2.0	2.2	-7.4
11	23	S X R	2.0	2.1	-6.5
21	5	S X R	2.0	2.1	-4.8
Bottom 8 hybrids:					
24	18	S XS	6.5	2.5	163.4
7	13	R x MS	4.5	1.6	177.9
9	15	R X R	3.0	1.1	180.6
9	13	R x MS	4.5	1.6	181.6
2	9	MR x R	3.5	1.2	184.1
10	24	R X MS	4.5	1.5	209.7
24	17	MS X R	4.5	1.3	240.3
24	16	MS XMR	5.5	1.5	259.7

Ced2005 = Cedara in 2004/5 season; MP = mid parent

4.4   Discussion

Disease development was highly determined by environment (Table 4.5), thus disease occurrence and levels may differ drastically between years. However, in suitable environments, such as Cedara, onset of the PLS may be relatively early and development relatively strong, causing severe necrosis of large parts of the plant, reducing photosynthetic area and grain filling. On heavily infected plants, the ears often had few or no lesions, thus minimising the damage to the ear. The disease tended to have a rather uniform distribution within the trial at Cedara. At hot spot locations, such as Cedara, South Africa, screening for disease resistance can be effective, at least during years with favourable weather for disease development.

As from the literature, the disease was first observed at silking and quickly intensified. This late arrival in the crop cycle increased the chances that the disease intensity remains low. At Cedara, in South Africa, the first lesions were observed on the top leaves (above the ear) in some entries but in most entries on the lower leaves. This suggests that the fungus is mainly spreading from the soil to the lower leaves of the host, but that there is also an airborne spread. Disease scores could be classified into

seven classes. High levels of resistance were encountered in K, SC, N3 and CIMMYT B heterotic groups, indicating that hybrids between lines drawn from these groups would show high levels of resistance. Resistance was not only expressed by reduced severity but also by reduced number and size of lesions.

Although PLS may still be regarded as a minor disease it might follow a similar trend to GLS in the USA, which appeared in 1925, but only became an economic disease in the 1970's (Huff *et al*., 1988). However, the significant variation among hybrids and inbreds for resistance to PLS provides breeders with an opportunity for selection. Low PLS rating scores for 34% of the hybrids and 37% of inbreds that were classified resistant, indicated that there were high levels of resistance to PLS in this set of germplasm, for breeding in Southern Africa. Consequently, scientists do not have to look beyond the region in search of resistance, which is obtainable in these local and adapted inbred lines with base line resistance to other regional diseases. The inbreds B23, 9314085P, B17 and CML444, which contributed significant levels of resistance in the most resistant hybrids, would be recommended for use as breeding sources in regional programmes that aim at enhancing resistance. In addition, the single cross hybrids with high levels of resistance would be recommended for developing new PLS-resistant lines through pedigree breeding. However, the following experimental hybrids, which combined high levels of resistance with high relative yield ranging from 121 to 141% of the overall mean yield would be recommended for further testing and subsequent release for cultivation in disease prone areas: CML444/A26 (141.9%), B20/CML488 (138%), B19/CML488 (124%) and B21/CML395 (121%). High level of resistance observed in this study was in agreement with that observed in a previous study conducted by Flett (2004) who reported a low range of PLS scores and concluded that commercial hybrids had high levels of resistance in South Africa.

General combining ability accounted for 90%, while SCA explained only 10% of sum of squares for the crosses (hybrids), indicating that additive effects played a greater role than their non-additive counterparts in conditioning resistance to PLS. These results were consistent with previous studies in Brazil and the USA where GCA has been reported to be predominant over SCA effects in conferring resistance (Pegoraro *et al*., 2002; Carson, 2001). Although Vivek *et al*. (2001) reported that both GCA and SCA for PLS were highly significant in regional maize, GCA accounted for 65% of the genetic variation. In the current study, female GCA accounted for 58% and male GCA effects 32%, implying a possible role of maternal effects in controlling resistance in hybrids. Hallauer and Miranda (1988) reported that if present maternal effects would inflate the

GCA or the additive variance. Future studies of PLS inheritance should employ models that include reciprocal effects in order to investigate the "speculative" role played by maternal effects in influencing resistance in this set of maize germplasm.

Highly significant (P<0.01) environmental effects could be explained by differences in the level of disease pressure at Cedara (mean rating 2.9) and RARS (mean = 2.1) in 2004/5. Deep conventional tillage is a traditional practice at RARS, which reduces the level of disease inoculum in and on the ground as infected debris is ploughed deep under every season. At Cedara, trials were grown in the same field that had been continuously planted to maize following reduced tillage practice. Furthermore, Cedara has a favourable environment for PLS development, which is characterised by humid, morning dew and is situated within the mist-belt of KwaZulu-Natal Midlands. A significant (P<0.01) interaction of female GCA with environment suggested that selection progress would be impeded because different sets of hybrids would be selected in each environment. Alternatively different set of female parents have to be used depending on the target environment. Vivek *et al*. (2001) also reported highly significant (P<0.01) GCA x environment effects in regional maize.

High levels of heritability estimates (52 to 73%) were a reflection of high GCA variance relative to the residual. High levels of heritability estimates (55 to 85%) have been reported in previous studies (Carson *et al*., 2005; Carson, 2001; Vivek *et al*., 2001). The high levels of heritability estimates suggested that simple selection procedures based on GCA would be employed to enhance resistance in regional cultivars. The significant (P<0.01) parent on offspring regression (0.62) also indicated that resistance of hybrids would be predictable from the levels of resistance levels in their parents. The study also showed that most resistant hybrids were formed between resistant lines (R x R or R x MR), indicating that resistant single cross hybrids would be obtained when both parents have high levels of resistance. This confirmed the predominance of additive effects, because resistance was diluted in hybrids when a resistant parent was crossed to a susceptible parent. The observation that there were hybrids that combined high levels of resistance and high yield indicated that breeding for resistance would not necessarily compromise grain yield in this set of germplasm.

4.5   Conclusion

This study was conducted to determine (a) levels of resistance, (b) heritability and (c) gene action conditioning resistance in a selected set of Southern African maize. Disease

scores could be classified in seven statistical classes. Resistance was not only expressed by reduced severity but also by reduced lesion number and or reduced lesion size. The results revealed high levels of resistance for the hybrids (34%) and inbred lines (37%). Resistance was highly heritable (62 to 73%). Additive gene action played a predominant role, while non-additive effects were not significant in conditioning resistance. Future studies should employ models which include reciprocal effects to investigate the "speculative" role of maternal effects in influencing resistance in this set of Southern African maize. Several experimental hybrids combined high levels of resistance with high relative grain yield (121 to 141% of overall trial mean): CML444/A26 (141.9%), B20/CML488 (138%), B19/CML488 (124%) and B21/CML395 (121%). These would be recommended for further testing and subsequent release. In addition, the inbred lines B23, B17, B12 and CML444, which showed significant contribution of PLS-resistance to the hybrids would be recommended for use as breeding sources.

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Appendices

Appendix 1. Sets of crosses formed in a North Carolina II Mating design (Principal Selection criteria: D = drought tolerance, C = conventional, G = GLS resistance); Conventional means lines were selected on the basis of high yield potential only.

Set 1: Grey Leaf Spot X Drought Tolerant Crosses

♀/♂	CML442 (D)	CML312 (D)	CML445 (D)
B16 (G)			
B17 (G)			
B18 (G)			

Set 2: Conventional X Drought Tolerant Crosses

♀/♂	CML395 (D)	CML444 (D)	CML488 (D)
B19 (C)			
B20 (C)			
B21 (C)			

Set 3: Drought Tolerant X Conventional Crosses

♀/♂	A7 (C)	A8 (C)	A9 (C)
CML442 (D)			
CML312 (D)			
CML445 (D)			

Set 4: Drought Tolerant X Conventional Crosses

♀/♂	CML489 (C)	A26 (C)	CIM24 (D)
CML395 (D)			
CML444 (D)			
CML488 (D)			

Set 5: Conventional X Grey Leaf Spot Resistant Crosses

♀/♂	A13 (G)	A14 (G)	A15 (G)
A7 (C)			
A8 (C)			
A9 (C)			

Set 6: Conventional X Grey Leaf Spot Tolerant Crosses

♀/♂	B16 (G)	B17 (G)	B18 (G)
B22 (C)			
B23 (C)			
B24 (C)			

Set 7: Grey Leaf Spot X Conventional Crosses

♀/♂	B19(C)	B20(C)	B21(C)
A13 (G)			
A14 (G)			
A15 (G)			

Set 8: Conventional X Conventional Crosses

♀/♂	B22(C)	B23(C)	B24(C)
K64R (C)			
B11 (C)			
B12 (C)			

Appendix 2: PLS Resistance and yield of 80 hybrids in 2004/5 season

ENTRY	NAME	PLS Score		Yield	
		Cedara	RARS	(t/ha)	% Mean
32	CML444/A26	1.6	1.1	8.4	141.9
17	B20/CML488	1.9	1.0	8.2	138.3
31	CML395/A26	2.4	1.0	7.5	127.7
42	A9/A14	2.4	1.1	7.4	124.9
16	B19/CML488	2.0	1.1	7.3	124.4
1	B16/CML442	2.3	1.0	7.3	123.2
12	B21/CML395	1.4	1.6	7.1	121.1
75	**SC633**	2.9	2.3	7.1	120.7
39	A9/A13	4.4	1.5	7.0	119.0
54	B24/B18	6.3	5.5	6.9	117.5
58	A13/B20	2.3	1.1	6.9	117.5
55	A13/B19	2.4	1.1	6.9	117.1
63	A15/B21	2.3	1.5	6.9	116.5
18	B21/CML488	1.6	1.1	6.9	116.4
37	A7/A13	4.8	1.1	6.8	115.5
11	B20/CML395	1.9	1.0	6.8	114.6
2	B17/CML442	2.0	1.0	6.7	113.7
43	A7/A15	2.8	0.9	6.7	113.5
13	B19/CML444	1.8	1.0	6.6	111.6
29	CML444/CML489	1.3	1.6	6.6	111.6
7	B16/CML445	2.4	2.5	6.6	111.2
15	B21/CML444	2.3	1.0	6.5	110.9
66	B12/B22	1.3	1.1	6.4	109.3
45	A9/A15	3.3	1.1	6.4	108.8
4	B16/CML312	1.7	0.9	6.4	107.9
21	CML445/A7	4.7	4.4	6.4	107.9
56	A14/B19	3.6	1.6	6.4	107.6
30	CML488/CML489	2.0	1.0	6.3	106.9
50	B23/B17	2.2	0.9	6.3	106.3
10	B19/CML395	1.9	1.0	6.3	106.2
61	A13/B21	3.0	1.5	6.3	106.0
34	CML395/CIM24	2.1	0.8	6.2	105.8
51	B24/B17	4.5	2.6	6.2	105.8
25	CML442/A9	3.5	2.0	6.2	105.7
59	A14/B20	1.7	1.0	6.2	105.4
8	B17/CML445	2.5	3.0	6.2	105.3
46	B22/B16	1.4	1.1	6.2	104.5
28	CML395/CML489	2.2	0.9	6.2	104.5
36	CML488/CIM24	1.4	1.1	6.1	104.2
33	CML488/A26	2.3	2.0	6.1	103.1
40	A7/A14	3.2	2.0	5.9	100.3
57	A15/B19	2.3	1.1	5.8	98.9
3	B18/CML442	5.1	1.5	5.8	98.1
27	CML445/A9	2.9	3.5	5.8	97.6

*Standard hybrids are in bold.

Appendix 2. PLS resistance and yield of hybrids at Cedara and RARS in 2004/5 (Continued)

Entry	Name	PLS Sore		Yield	
		Cedara	RARS	t/ha	%Mean
38	A8/A13	4.9	1.4	5.7	96.8
5	B17/CML312	2.0	1.0	5.7	96.2
24	CML445/A8	5.1	5.9	5.7	96.1
68	B11/B23	2.0	1.0	5.7	95.9
35	CML444/CIM24	2.0	0.9	5.6	95.1
41	A8/A14	3.0	2.0	5.5	94.0
14	B20/CML444	1.4	1.0	5.5	93.6
22	CML442/A8	4.2	0.9	5.5	93.5
44	A8/A15	4.4	2.0	5.4	91.8
47	B23/B16	0.8	1.1	5.4	91.3
69	B12/B23	0.8	3.4	5.4	91.2
73	**SC513**	7.1	4.6	5.4	91.1
76	**SC627**	3.2	1.4	5.4	90.9
65	04C2181	1.4	2.5	5.3	89.1
48	B24/B16	5.6	2.4	5.2	88.2
70	K64R/B24	4.5	4.0	5.2	88.2
72	04C2182	1.4	5.5	5.2	88.2
53	B23/B18	2.2	1.1	5.2	87.7
62	A14/B21	2.9	3.1	5.2	87.3
6	B18/CML312	4.6	3.0	5.1	86.4
78	**ZS255**	5.9	6.6	5.0	85.4
74	**SC403**	5.1	7.0	5.0	85.1
77	**ZS257**	2.5	4.0	5.0	84.6
19	A7	2.1	1.0	4.9	83.1
23	CML312/A8	2.3	2.5	4.9	83.1
64	04C2179	1.7	1.4	4.8	81.5
60	A15/B20	1.7	1.0	4.8	80.7
49	B22/B17	1.2	1.0	4.5	76.4
79	**R201**	1.5	3.1	4.4	75.3
80	**R215**	1.9	2.0	4.4	75.1
67	K64R/B23	1.1	1.1	4.4	74.3
20	CML312/A7	2.8	1.6	4.4	74.0
52	B22/B18	3.7	1.1	4.4	74.0
26	CML312/A9	3.7	3.0	4.1	70.3
9	B18/CML445	3.0	3.4	4.0	67.9
71	B11/B24	4.1	6.5	3.8	64.1
MEAN		2.9	2.1	5.9	100
SED		0.6	0.6	0.6	
LSD $_{0.05}$		1.7	1.7	1.6	

Standard hybrids are in bold.

Chapter 5: Grain Yield Evaluation of Southern African Maize Base Germplasm under Drought Stress

Abstract

Frequent drought causes devastating yield losses in maize with serious implications for food security in Southern Africa, yet only a few studies have evaluated yield under drought stress in regional germplasm. The objective of this study was therefore, to evaluate: a) grain yield b) levels of stress tolerance; c) gene action; d) heritability; e) combining ability; and f) correlations between yield and secondary traits under drought stress. Twenty-seven lines were mated in eight sets using North Carolina Design II scheme, resulting in 72 hybrids. Experimental hybrids and eight standard hybrids were evaluated in an 8 x 10 α-lattice design with two replications across six environments, under random drought and managed drought stress at flowering during 2004 to 2005. Results showed highly significant variation among hybrids for drought stress tolerance, yield potential and secondary traits associated with yield. There were high levels of general combining ability (GCA) for yield (72%), number of ears per plant (77%), anthesis-silking interval (77%), flowering dates (85 to 93%) and other traits (65 to 93%). Thus, predominantly additive gene action conditioned hybrid performance under drought stress, suggesting that in forming hybrids both parents should carry high levels of drought tolerance. Estimates of heritability for yield decreased from 60% under well-watered conditions to 19% under drought stress at flowering. Heritability estimates of anthesis-silking interval (ASI) and number of ears per plant (EPP) showed a reverse trend. The ASI and EPP were significantly correlated with yield and both had moderate heritability (42%) under drought stress. Fifteen experimental hybrids and one standard were drought tolerant. The following hybrids, which combined high yield potential (≥120% of overall mean yield) and high drought tolerance, would be considered for release: 04C2179 (121.6%), CML444/CIM24 (119.6%), A14/B19 (127.5%), CML445/A7 (120.1%) and B17/CML445 (124.8%). Lines CML442, CML488, CML312, A14, A8 and B21 had high positive GCA effects for yield under drought stress. These lines would be recommended for use as breeding sources. It can be concluded that hybrids combining high yield potential and high drought tolerance would be obtainable.

Keywords: Drought Stress Tolerance, GCA, Grain Yield, Heritability, Maize, SCA

5.1   Introduction

Maize is essential in Southern Africa for food security and regional economic stability, because the crop sustains both household livelihoods and the national economies. However, adequate grain production is hampered by frequent drought among other constraints (Zaidi *et al*., 2004). In addition to threatening food security, drought has serious ripple effects on the whole economy, because these economies are dependent on agriculture. For example, Richardson's (2005) study showed that rainfall amount had a positive correlation (r =0.65) with gross domestic product in Zimbabwe over 53 years. Above world average grain yield losses due to frequent drought occur in the sub-continent. Rosen and Scott (1992) reported reduction of grain production amounting to 60% in Southern Africa as compared to a global average of 17% (Edmeades *et al*., 1992).

Drought is generally difficult to control as compared to other constraints such as low soil fertility, especially due to its unpredictability over time and space. Campos *et al*. (2004) reported that soil moisture levels vary between years and within fields even on the same farm. A practical option, especially for resource-constrained small-scale farmers, who have no or limited access to irrigation facilities, is to breed cultivars that can withstand drought stress without incurring a yield penalty when they are grown under non-stressful conditions. Breeding should be targeted at releasing cultivars with drought stress tolerance at flowering, because tassel emergence and ear formation have been reported to be critical stages that are associated with highest grain yield loss (Cakir, 2004). Progress in breeding such cultivars has been slow due to reduced heritability of grain yield under abiotic stress, caused by high cultivar x environment (G x E) interactions. According to Banziger *et al*. (2004), high G x E was a result of variation in timing and severity of water deficit, genetic variation at flowering, and nutrient deficiencies interacting with water deficit.   High error variances have also been caused by variable plant stand and variability in soil water holding capacity (Banziger *et al*., 2004). Studies have found that higher genetic gains can be realised when selection is conducted based on stress at flowering rather than at grain filling (Bruce *et al*., 2002). In addition it was long ago reported that grain yield was highly correlated with anthesis to silking interval (ASI) and number of ears per plant in tropical populations, especially when drought stress occurs at flowering (Chapman and Edmeades, 1999; Edmeades *et al*., 1993). Banziger *et al*. (2000) have suggested the use of indirect selection for grain yield by targeting ASI and

number of ears per plant (EPP). These traits are easy to measure and are less affected by G x E under drought stress.

Knowledge of gene action conditioning grain yield under low moisture stress in base germplasm would assist scientists in devising appropriate breeding strategies. Betran *et al*. (2003) reported additive effects were predominant in controlling grain yield in tropical maize under drought stress. Studies of quantitative trait loci have found that additive gene action controlled flowering traits, whereas dominance effects explained grain yield, ASI and EPP (Agrama and Moussa, 1996; Guei and Wassom, 1992). A literature survey showed few published research of genetic effects conditioning grain yield under drought stress in Southern African maize. Use of local materials would increase breeding progress, because these already have adaptation to other important yield limiting factors such as maize streak virus disease. Moreover, genetic information specifically applies to the specific germplasm and environments in which materials were tested (Falconer, 1981). Thus information generated from other regions or germplasm may not have a direct application to Southern African. The objective of this study was to investigate genetic effects that influence grain yield of Southern African maize base germplasm under abiotic stress. Specifically the study tested the following: (a) levels of drought stress tolerance; (b) gene action conditioning grain yield and associated traits; (c) heritability of grain yield and associated traits; (d) correlation between grain yield and secondary traits; (e) relationship between testing environments; and (f) combining ability effects estimates of inbred lines for grain yield under low drought stress conditions.

5.2   Materials and Methods

5.2.1 Germplasm

The lines used in this study consisted of a sample drawn from the following heterotic groups and their derivatives (Table 5.1): a) International Maize and Wheat Improvement Centre (CIMMYT): A and B; and b) regional: P, K64R, SC, N3, I, M. Detailed descriptions of these germplasm groups have been presented (Mickelson *et al*., 2001; Gevers and Whythe, 1987). They represented three classes: drought tolerant, conventional and GLS resistant (Table 5.1). Twelve inbred lines comprising six drought-tolerant and six gray leaf spot resistant inbreds were obtained from CIMMYT-Zimbabwe, and 15 conventionally bred lines were obtained from Seed Co. These inbred lines were specifically adapted to tropical environments in east and

Southern Africa. Inbred lines were divided into eight sub-groups of three each, according to their background. Three inbred lines in one set were used as females and crossed with three inbred lines from a second set used as males, according to a North Carolina Design II Mating Scheme (Robinson and Comstock, 1948, 1952; Hallauer and Miranda, 1988). Each inbred line was used once as a female parent in one set and once as a male parent in another set, except the inbreds K64R, B11 and B12, which were used as females only, and CML489, A26 and CIM24, which were used as male parents only (Appendix 1, in sets 8 and 4, respectively). The inbreds CML489, CIM24 and A26 replaced K64R, B11 and B12 as male in set 4, which had failed to provide adequate pollen due to severe attack by maize streak virus. Consequently, eight sets of hybrids comprising nine hybrids each were formed among the 27 inbreds. The 27 inbred lines, their 72 crosses and eight standard hybrids effectively constituted the reference population for the study. The eight standard hybrids used in the study were drought tolerant hybrids (R201, R215, ZS255 and SC403) and four other hybrids (SC633, SC513, SC627 and ZS257) that were widely grown in the area.

Table 5.1: Parent inbred lines used in a North Carolina Design II mating

Designation	Heterotic Group	Principal Selection Criteria	Principal Selection Class
CML442	A	Drought Tolerance	1
CML312	A	Drought Tolerance	1
CML445	AB	Drought Tolerance	1
CML395	B	Drought Tolerance	1
CML444	B	Drought Tolerance	1
CML488	B	Drought Tolerance	1
A7	M	Conventional	2
A8	M	Conventional	2
A9	P	Conventional	2
K64R	K	Conventional	2
B11	K	Conventional	2
B12	K	Conventional	2
A13	A	GLS Resistance	3
A14	A	GLS Resistance	3
A15	N	GLS Resistance	3
B16	I	GLS Resistance	3
B17	B	GLS Resistance	3
B18	B	GLS Resistance	3
B19	K	Conventional	2
B20	KB	Conventional	2
B21	K	Conventional	2
B22	S	Conventional	2
B23	S	Conventional	2
B24	S	Conventional	2
CML489	AB	Conventional	2
A26	I	Conventional	2
CIM24	A	Drought Tolerance	1

5.2.2 Experimental Design

Grain yield of 72 experimental hybrids, 8 standard hybrid checks and 30 parent inbred lines was evaluated under managed drought stress at flowering and random moisture stress, in Zimbabwe during the winter of 2004 and summer of 2004/5, respectively (Table 5.2). Hybrid trials were conducted in an 8 x 10 α-lattice designs with two replications, whereas inbred line trials were laid out as 5 x 6 α-lattice designs with three replications. Inbreds were evaluated under moisture stress at Save Valley (SV) and under well-watered conditions at Kadoma Research Centre (KRC), during the winter of 2004 and summer of 2004/5, respectively. In addition, to location-year combination, environments were created by varying watering regimes and plant population density (Table 5.2) as suggested by Eberhart and Russell (1966). Fertiliser was applied at the rate of 166 N, 28 P and 17 K (kg/ha) at KRC; and at Save Valley the fertiliser rate was 104 N, 18 P and 11 K (kg/ha). Standard cultural practices, which included hand weeding, were followed at both stations.

5.2.3 Managed Drought Stress Experiments

Moisture stress experiments were established at Save Valley (SV), during the rain-free winter of 2004 (Table 5.2). These trials were established and managed according to procedures developed by CIMMYT (Banziger *et al*., 2000). This managed drought experiment was a simulated drought condition that was created at the most drought susceptible flowering stage. The study was conducted in winter to avoid disruption of the experiment by natural rainwater during summer. The fields used for the experiments were level beds with uniform sandy loam soils. Further control for soil variation was achieved by laying the experiment as an α-lattice block design such that the block was divided into 10 sub-blocks with eight plots each. At planting, eight hybrids were planted in each sub-block (incomplete blocks) after a randomisation of the hybrids in Genstat. A control experiment was established at the same site but in a separate block 120 m away and was managed in the same way as the stressed block except that it was irrigated throughout the season. The control experiment (SV2004HP) had the same plant population density as the stressed experiment (Table 5.2). An additional well-watered experiment (SV2004LP) was established at a lower planting density (26 000) in order to investigate whether density effects provide additional stress during drought screening (Table 5.2).

Adequate irrigation (±100% of field capacity) was applied in all experiments from planting and throughout the vegetative phase, using sprinkler irrigation system. Based on the previous data of the standard hybrids (R201, R215, SC403 and ZS255) at this site, the average pollination date for the trial was predicted. In addition, the crop was monitored on a daily basis. Irrigation was then discontinued in the stress block, when 5% of the standard hybrids had emerged tassels, which coincided with 60 days from planting. Irrigation continued in the control blocks. The irrigation was applied such that all the sub-blocks were maintained at field capacity. Soil moisture level was determined daily, using a calibrated neutron probe (Model: Didcot Instruments). At 50% pollen shedding, the soil moisture had declined to 50% of field capacity at the 90 cm depth. By the end of the grain filling period, the soil moisture was at 26% of the field capacity in the effective rooting zone (0 to 45 cm depth), and 35% at 90 cm depth.

5.2.4 Random Drought Experiments

Two experiments were established at SV and one at KRC, which represented random drought prone sites in tropical lowland dry and mid altitude dry environments, respectively (Table 5.2), during the summer of 2004/5. Random drought occurred at both locations during pollination and grain filling stages and no diseases were observed.

Table 5.2. Features of environments used in the drought screening of hybrids in 2004 to 2005

Environment Code	Location	Season	Type of Environment	Plant Density	Mean yield	Altitude (m)
KRC2005H	Kadoma	2004/5	Random drought stress	44 000	2.699	1149
SV2004MS	Save Valley	2004	Managed drought stress at flowering	53 000	2.826	556
SV2005H	Save Valley	2004/5	Random drought stress	53 000	2.760	556
SV2005LP	Save Valley	2004/5	Random drought stress	22 000	2.582	556
SV2004H	Save Valley	2004	Well watered at high plant density	53 000	6.523	556
SV2004LP	Save Valley	2004	Well watered at low plant density	26 000	5.277	556

5.2.5 Data Collection

Field data were collected on a whole plot basis following standard procedures used by CIMMYT (Banziger *et al*., 2000; CIMMYT, 1985). Grain yield and secondary traits such as anthesis-to-silking interval (ASI) and number of ears per plant which have

been used in determining maize tolerance to moisture stress (Betran *et al*., 2003; Banziger *et al*., 2000) were measured in all trials. Number of days to anthesis (DMP) and silking (DMS) were measured as number of days from planting to when 50% of the plants were shedding pollen and had silks of at least 5 cm emerged, respectively. Anthesis to silking interval (ASI) was then calculated using the formula:

$$ASI\ (days) = DMS - DMP$$

Plant and ear height (cm) were measured as the distance from the base of the plant to the height of the first tassel branch and the node bearing the upper ear, respectively. Plant aspect was rated on a scale of 1 to 5, where 1 = excellent overall phenotypic appeal and 5 = poor overall phenotypic appeal. Leaf rolling was rated using a score of 1 = unrolled, turgid, and 5 = leaf rolled like onion leaf; while leaf senescence was rated on a 1 to 10 scale, where 1 = 10% dead leaf area and 10 is 100% dead leaf area. Ear aspect was scored on a 1 to 5 scale, where 1 = clean, uniform, large and well-filled ears, and 5 = rotten, variable, small, and partially filled ears. At harvest all ears from each plot were counted and the number ears per plant (EPP) were calculated using the formula: EPP = EC/PC, Where EC and PC = number of ears and number of plants per plot, respectively.

All ears were bulked, shelled and grain weight measured. Grain yield (t/ha) was computed from shelled grain weight per plot adjusted to 12.5% moisture content, using the formula:

Grain Yield (t/ha) = [Grain Weight (kg/plot) x 10 x (100-MC)/ (100-12.5)/(Plot Area)], where MC = Grain Moisture Content.

Drought stress tolerance for individual hybrids was determined by calculating a relative drought tolerance (RDT) value based on theoretical aspects of selection under stress and non-stress conditions (Tollenaar and Lee, 2002; Rosielle and Hamblin, 1981) using the formula:

$$RDT\ (\%) = 100 \times (Y_{MS}/Y_C),$$

Where Y_C = hybrid yield under well watered or control conditions, and Y_{MS} = hybrid yield under carefully managed moisture stress conditions at flowering. High relative drought tolerance value would indicate that the hybrid is tolerant to moisture stress, relative to its yield potential as measured under well-watered conditions. According to Rosielle and Hamblin (1981), selection for tolerance to stress is equivalent to selection for low yield depression under stress.

Mid parent heterosis (MPH) for grain yield was calculated for each hybrid under moisture stress conditions (Falconer, 1989) using the formula:

$$MPH\ (\%) = (F_1 - MP)/MP \times 100,$$

Where F_1 = mean of the F_1 hybrid performance, MP = mean of two parents making the cross using the formula: $(P_1 + P_2)/2$, where P_1 and P_2 are the means of the inbred parents.

5.2.6 Statistical Analyses

General analyses of variance for lattice block designs (Cochran and Cox, 1960) for the inbred and hybrid data including standard checks within individual environments were performed using IRRISTAT Computer software (International Rice Research Institute, 2003). Additive Main Effects and Multiplicative Interaction (AMMI) Analysis (Crossa *et al.*, 1990) was conducted using GenStat (2003) computer software to predict the grain yield means for the hybrids across the four drought stress environments. According to Crossa *et al*. (1991), the AMMI model removes residual or noise variation from cultivar x environment interaction (i.e., G x E) by using information from other environments to refine the estimates within a given environment, whereas the lattice block designs would only make intra-location adjustments.

Genetic analyses for grain yield and its associated data were performed in SAS (SAS Institute, 1997) as a fixed effects model for experimental hybrids (excluding standard hybrids), as described by Hallauer and Miranda (1988) for across environments using the following linear model:

$Y_{ijkpq} = \mu + S_p + gi(S_p) + g_j(S_p) + h_{ij}(S_p) + E_q + r_k(SE)_{pq} + (ES)_{pq} + (Eg)_{iq}(S_p) + (Eg)_{jq}(S_p) + (Eh)_{ijq}(S_p) + e_{ijkpq}$

Where i =1, 2, 3; j = 1, 2, 3; k = 1, 2; p = 1, 2, 3, 4, 5, 6, 7, 8; q = 1, 2, 3, 4, 5, 6 and Y_{ijkpq} denotes the value of the hybrid of a mating of the ith female line, the jth male line, in the kth block, within set p and in the qth environment. The terms are defined as follows:

μ = Grand mean

S_p =   the average effect of the pth set

$gi(S_p)$ = the GCA effect common to all hybrid of the ith female line nested within p^{th} set,

$g_j(S_p)$ = the GCA effect common to all hybrid of the jth male line nested within p^{th} set,

$h_{ij}(S_p)$ = the SCA effect specific to hybrid of the ith female and j^{th} male line nested within p^{th} set,

E_q = average effect of qth environment

$r_k(SE)_{pq}$ = the effect of the kth replication nested within the pth set and q^{th} environment

$(ES)_{pq}$ = the interaction between set effects and the environment

$(Eg)_{iq}(S_p)$ and $+(Eg)_{jq}(S_p)$ = the interaction between environment and GCA nested within sets

$(Eh)_{ijq}(S_p)$ = the interaction between environment and SCA nested within sets

e_{ijkpq} = the random experimental error

According to Hallauer and Miranda (1988), the main effects due to females (sets) and males (sets) are equivalent to the GCA (general combining ability), while male x female (sets) interaction effects represent the SCA (specific combining ability) effects in a diallel mating. General combining ability and SCA effect estimates for inbred lines and crosses, respectively, were determined by line x tester analysis in Agrobase (2005) computer package. Using the variance ratios in REML (GenStat, 2003), heritability estimates were calculated as suggested by Hallauer and Miranda (1988) for the fully inbred parents (F = 1) (where $\sigma^2_m = \sigma^2_f = \frac{1}{2}\sigma^2_A$; and $\sigma^2_{mf} = \sigma^2_D$) using the formulae:

a) $h^2 = 2\sigma^2_m/(\sigma^2/r + \sigma^2_{mf} + 2\sigma^2_m)$ for one environment, and

b) $h^2 = 2\sigma^2_m/(\sigma^2/re + \sigma^2_{fme}/e + 2\sigma^2_{me}/e + \sigma^2_{mf} + 2\sigma^2_m)$ for across environments,

Where σ^2_m = male (set) variance, σ^2 = random error variance; σ^2_{mf} = male x female (set) variance; σ^2_{fme} = environment x male x female (set) variance; σ^2_{me} = environment x male (set) variance; r = number of replications and e = number of environments. σ^2_f = female (set) variance; σ^2_A = additive variance and σ^2_D = dominance variance.

Heritability estimates were calculated using the male variance (σ^2_m) to avoid the upward bias of heritability due to maternal effects if the female variance (σ^2_f) was used. In addition, phenotypic correlations were performed between grain yield and other agronomic traits and among test environments using GenStat (2003) computer software.

5.3   Results

5.3.1 Performance of Inbred Lines

Inbreds differed significantly for grain yield, EPP, ASI, DMP, leaf rolling (LR) and leaf senescence (LS) (Table 5.3). Grain yield ranged from 2.1 (B24) to 5.3 t/ha (A8) under well watered and from 0.2 (CML312) to 3.6 t/ha (CIM35) under moisture stress. Inbred lines K64R and B17, which belonged to the conventional and GLS resistance classes, respectively, out-yielded all entries in the drought tolerant class. The highest yielding entry in the drought class was CML445 with a yield under 2.2 t/ha. Anthesis

to silking interval ranged between -4.3 (CML444) and +2.7 (B19) under well watered, and from –2.0 to 10.5 days under moisture stress, while EPP ranged between 0.8 (B24) and 1.6 (CML489) under well watered, and from 0.1 (CML489) to 1.5 (A8) under moisture stress. Inbred lines K64R and B12 had the shortest ASI under moisture stress, while anthesis ranged from 61 (B21) to 73.7 days (CML444) under well watered, and from 89.5 to 103 days under moisture stress. The LR and LS scores ranged from 1.0 to 3.5, and from 1.0 (CML489) to 8.5 (A7).

Table 5.3. Performance of Inbred Lines under well-watered (KRC) and moisture stress conditions (SV)

Inbred	Grain Yield (t/ha)		Anthesis (days)		ASI		EPP		LS	LR
	KRC	SV	KRC	SV	KRC	SV	KRC	SV	SV	SV
	(WW)	(MS)	(WW)	(MS)	(WW)	(MS)	(WW)	(MS)	(MS)	(MS)
K64R	3.3	3.3	66.4	93.0	-1.3	-2.0	1.0	0.9	3.0	3.0
B17	4.3	2.8	65.6	101.0	-2.7	0.5	1.2	0.7	3.5	2.5
B16	3.9	2.5	67.6	97.5	0.3	1.5	1.1	0.8	3.5	2.5
B20	4.2	2.4	68.0	92.0	0.3	3.0	1.1	0.8	2.0	2.0
A7	5.2	2.2	68.9	95.0	0.3	1.0	1.1	0.9	8.5	3.0
B19	4.0	2.2	66.2	94.5	2.7	1.5	1.1	0.7	3.0	3.0
CML445	3.2	2.2	67.0	96.5	-0.7	0.5	1.0	0.9	1.5	3.0
A15	2.4	2.1	67.5	101.0	1.0	1.0	1.0	0.7	3.0	2.5
CML488	4.1	1.8	66.2	93.0	-1.3	3.0	1.2	0.9	5.0	3.5
A26	3.3	1.6	66.4	--	0.3	--	1.0	--	--	--
RSA414P	3.2	1.6	66.2	--	2.0	--	1.0	--	--	--
B23	3.2	1.6	70.4	--	-1.0	--	1.1	--	--	--
B22	2.8	1.6	68.1	--	-1.0	--	0.9	--	--	--
A8	5.3	1.4	72.2	101.5	-1.7	4.0	1.4	1.5	5.5	3.5
CML444	3.5	1.4	73.7	100.0	-4.3	1.5	1.0	0.8	5.0	2.5
B12	3.3	1.4	66.1	97.2	-1.0	-0.3	1.1	1.0	1.1	2.9
B18	2.5	1.4	72.5	99.0	1.0	3.0	0.9	0.8	3.5	3.5
A14	2.3	1.3	69.2	89.5	1.0	2.5	0.9	0.7	3.0	2.5
B21	3.0	1.0	61.3	91.0	--	4.5	0.9	0.5	2.0	2.5
A9	3.6	0.8	65.7	92.2	1.0	1.7	0.9	0.7	2.1	2.9
CML442	2.7	0.8	69.7	99.0	0.0	4.0	0.9	0.6	5.5	3.5
A13	3.1	0.7	68.2	98.0	0.0	5.0	1.0	0.5	4.0	2.5
B24	2.1	0.7	70.1	103.0	0.7	5.0	0.8	0.5	3.5	3.5
CML489	4.4	0.6	69.7		-1.3	2.1	1.6	0.1	1.0	1.0
CIM24	3.3	0.6	72.4	97.0	-2.3	2.0	1.0	0.5	5.5	3.5
CML395	3.7	0.4	70.3	99.0	-0.7	10.5	0.9	0.4	5.5	2.5
CML312	3.2	0.2	69.0	94.0	0.7	---	1.0	0.2	4.5	3.0
MEAN	3.40	1.65	68.10	96.06	-0.20	2.56	1.00	0.73	3.73	2.91
SED (5%)	0.30	0.56	0.40	2.92	0.50	2.08	0.10	0.11	0.69	0.75

KRC = Kadoma Research Centre; SV = Save Valley; WW, MS = Well watered and moisture stress conditions, respectively; --- = Data not recorded; LS = Leaf senescence score; LR = Leaf roll score.

5.3.2 Performance of Hybrids

The AMMI analysis of variance showed highly significant (P<0.01) variation among hybrids and their interaction with environments for grain yield (Table 4). However, only the first principal component (IPCA1) of the interaction was significant (P<0.01).

The IPCA1 scores were positively correlated (r = 0.573) with grain yield under managed drought stress at flowering. The IPCA1 scores were negatively correlated with grain yield under well-watered (r = -0.653) and random drought stress (-0.543). Hybrids differed significantly for drought stress tolerance as RDT ranged between 13% for A13/B20 and 83% for B23/B16 (Appendix 2). The IPCA1 scores were positively correlated (r = 0.941) with RDT. Fig. 1 shows the frequency (%) distribution of 80 hybrids for RDT, while Appendix 2 presents the top most and least tolerant hybrids. Hybrids could be assigned to five distinct classes according to their RDT values: a) Tolerant (RDT ≥ 61%); b) Moderate Tolerance (RDT = 51 to 60%); c) Moderate Susceptibility (RDT = 41 to 50%); d) Susceptible (RDT = 31 to 40%); and e) Very Susceptible (RDT ≤ 30%). Fifteen experimental hybrids fitted into the tolerant classes (RDT = 56 to 83%) and their RDT values were significantly greater than the mean (44.4%) (Appendix 2). The following hybrids combined high yield potential (≥120% of the overall trial mean yield) and high drought tolerance indices, and would be considered for release: 04C2179 (121.6%), CML444/CIM24 (119.6%), A14/B19 (127.5%), CML445/A7 (120.1%) and B17/CML445 (124.8%) (Appendix 2). One standard hybrid, ZS255 was classified as moderately resistant (RDT = 56%), but the rest of the standard hybrids were relatively susceptible. Among standard hybrids ZS255 was highest yielding (120%). The most drought susceptible experimental hybrid (B24/B17) was among the lowest yielding hybrids. In addition, hybrids displayed significant levels of heterosis for yield ranging from -80.48 to 515% (CML488/CML489) (Appendix 2). The most drought tolerant hybrids B23/B16 and A13/B21 had negative heterosis for grain yield.

Table 5.4. AMMI analysis of variance for hybrids across four drought stress environments

Source	DF	SS	MS	F	F. Probability
Total	639	677.4	1.06		
Treatments	319	372	1.166	1.54	0.000
Cultivars	79	133.6	1.691	2.23	0.000
Environments	3	5.1	1.686	0.1	0.958
Block	4	65.5	16.387	21.59	0.000
Interactions	237	233.4	0.985	1.3	0.016
IPCA1	81	127	1.568	2.07	0.000
IPCA2	79	76.9	0.973	1.28	0.071
IPCA3	77	29.5	0.383	0.5	1.000
Residuals	0	0			
Error	316	239.9	0.759		

IPCA = interaction principal axes; Treatments = Environments + Cultivars + Interactions.
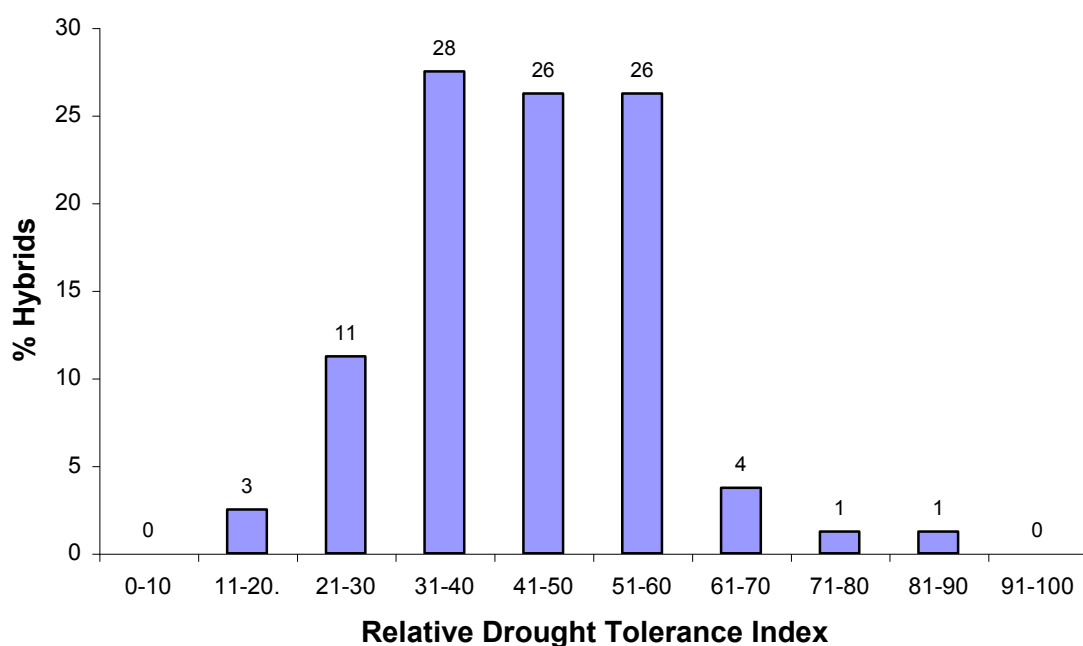
Fig. 1: Frequency distribution of 80 Hybrids for Drought Tolerance

5.3.3 Correlations between Grain Yield and Agronomic Traits

Grain yield had significant and negative correlation with ear aspect scores and ASI, and significant positive correlation with EPP for both hybrids and inbreds across moisture stress levels (Table 5). Correlations between hybrid grain yield and DMP and DMS were not significant under managed moisture stress or well-watered conditions, but they were not significant (r = -0.341) across environments. Correlation of grain yield with DMS was significant (P≤0.05) under moisture stress for the inbreds.

Table 5.5: Phenotypic Correlations between Grain yield and Agronomic traits

Trait	Hybrids			Inbreds	
	Moisture Stress	Well Watered	Across Environments	Moisture Stress	Well Watered
Anthesis-Silking Interval	-0.24	-0.24	-0.34 *	-0.45*	-0.22
Number of Ears/Plant	0.66 **	0.34 *	0.80 **	0.56 *	0.54 *
Anthesis (days)	0.06	0.06	-0.14	-0.02	-0.08
Silking (days)	-0.09	-0.09	-0.29	-0.36 *	-0.22
Ear Aspect (Score)	-0.74 **	-0.49 *	-0.86 **	-0.55 *	----
Leaf Roll (Score)	-0.25	---	---	-0.14	---
Leaf Senescence (Score)	---	--	---	-0.31	
Plant Height (cm)	---	--	0.06	--	0.06
Grain Moisture (%)	0.15	0.07	0.11	--	0.09

--- = Data not recorded; **, * = significant at P≤ 0.01 and P≤0.05, respectively.

5.3.4 Relationships among Environments

There was no significant correlation between hybrid ranks for managed moisture stress and well-watered environments (Table 5.6). Significant correlations were observed between random drought experiments and the well-watered low population environments.

Table 5.6: Correlation of hybrid ranks between the testing environments

Environment[+]	Well Watered	Managed Stress	KRC2005 (RMS)	SV2005H (RMS)	SV2005L (RMS)
Well-Watered (WW)	1.00				
Managed Stress	0.08	1.00			
KRC2005 (RMS)	0.23	0.09	1.00		
SV2005H (RMS)	0.36	0.15	0.17	1.00	
SV2005L (RMS)	0.32	0.25	0.31	0.26	1.00
SV2004 L (WWL)	0.32	0.47	0.79 *	0.43	0.59 *

[+]RMS = Random Moisture Stress; WWL = well-watered but with low population; KRC = Kadoma Research Centre in 2004/5; SV2005 = Save Valley in 2004/5; SV2004 = Save Valley in winter 2004; H = high population density and L = Low population density; * = Significant at P<0.05.

5.3.5 Heritability Estimates

Heritability estimates for grain yield ranged between 18% and 60 % under moisture stress and well-watered conditions, respectively (Table 5.7). Anthesis (87%) and silking dates (71%) had the highest heritability estimates across environments. Heritability varied from 18 to 63% for ASI, and from 7% to 45% for the number of ears per plant.

Table 5.7. Heritability (%) Estimates for Grain Yield and Agronomic traits across 6 environments

Trait	Environments[+]						Overall
	KRC2005 (RMS)	SV2005H (RMS)	SV2004M (MMS)	SV2005L (RMS)	SV2004L (WW)	SV2004H (WW)	
Grain Yield (t/ha)	33.7	24.2	18.8	31.3	60.6	21.2	53.7
ASI (days)	38.7	49.7	42.3	32.7	18.9	30.4	63.0
Ears/Plant (No.)	33.5	9.9	42.2	15.0	45.1	7.1	26.9
Anthesis (days)	75.9	63.0	48.8	68.1	71.3	58.6	87.1
Silking (days)	---	43.3	44.8	56.3	65.1	---	71.4
Plant Height (cm)	48.4	57.0	-	65.4	-	-	53.4
Ear Height (cm)	59.3	32.9	-	55.0	-	-	47.1
Ear Aspect (Score)	33.1	-	16.3	-	49.9	30.7	25.7
Grain Moisture (%)	---	34. 9	18.8	31.07	39.3	---	25.1

[+]RMS, MMS and WW, refer to random moisture stress, managed moisture stress and well-watered conditions, respectively; KRC = Kadoma Research Centre in 2004/5; SV2005 = Save Valley in 2004/5; SV2004 = Save Valley in winter 2004; H = high population density and L = Low population density; -- = Data not collected.

5.3.6 Gene Action

Environments and hybrid sets were highly significant (P<0.01) for grain yield and all other traits (Table 5.8). Interaction effects between environments and sets were also highly significant (P<0.01) for grain yield, ASI, number of ears per plant, grain moisture, but were not significant for ear aspect scores. General combining ability effects for male and female lines and SCA effects were also highly significant (P<0.01) for grain yield and most other traits. General combining ability effects were predominant over the SCA for grain yield (72%) and all agronomic traits (68 to 93%) (Table 5.9). Although SCA effects were almost negligible (6.94%) for DMP, 27% for grain yield, and 31% for grain moisture (Table 5.9).

Table 5.8: Mean squares for grain yield and agronomic traits across six environments.

Source	d. f	Yield (t/ha)	ASI (days)	EPP	DMS (days)	DMP (days)	Ear Aspect	Ear Height (cm)	Grain Moisture (%)	Plant Height (cm)
Environment (Env.)	5	556.4 **	65.03 **	154.37 **	21763.40 **	29834.46 **	24.53 **	81.62 **	377.76 **	173.34 **
Set	7	7.7 **	9.87 **	4.37 **	17.70 **	44.07 **	11.24 **	15.27 **	9.32 **	13.73
Env x Set	35	2.21 **	2.94 **	2.45 **	2.13 *	1.94 **	1.29	1.25	2.14 **	1.77 *
Rep/Set/Env.	48	3.21 **	0.91	2.03 **	1.75 **	2.18 **	0.94	0.86	0.81	1.07
Male/Set	16	4.15 **	7.18 **	2.62 **	23.54 **	32.40 **	3.13 **	6.72 **	2.90 **	8.93 **
Female/Set	16	3.59 **	3.93 **	5.11 **	17.55 **	25.81 **	5.71 **	13.43 **	2.56 **	14.42 **
Male x Female/Set	31	1.59 *	1.80 **	1.18	3.63 **	2.32 **	1.09	1.68 **	1.33	2.71 **
Env x Male/Set	85	1.42 **	1.55 **	1.84 **	2.45 **	1.84 **	1.27	1.44 *	1.78 **	1.60 **
Env x Female/Set	80	1.67 **	1.89 **	2.55 **	3.58 **	2.61 **	1.42	1.15	1.29 **	1.18
Env x Female x Male/Set	155	1.30 **	1.14	1.18	1.23 **	1.00	1.02	0.93	1.15	0.95

**,* = Mean Square Value Significant at 0.01 and 0.05, respectively; ASI = Anthesis to Silking interval (days); DMS = days to 50% silk emergence; DMP = Days to 50% pollen shed; Env. = Environment; EPP = Number of ears per plant

Table 5.9: Proportion (%) of the total genetic sum of squares for yield and agronomic traits

Trait	GCA			SCA
	Male	Female	Total GCA	
Grain Yield	39.8	32.4	72.2	27.8
Anthesis (Days)	53.2	39.9	93.1	6.9
Silking (Days)	50.4	35.3	85.7	14.2
Anthesis to Silking Interval (Days)	50.7	26.1	76.8	23.2
Number of Ears per Plant	27.3	50.2	77.5	22.5
Plant Height (cm)	33.3	50.5	83.8	18.4
Ear Height (cm)	30.0	56.4	86.4	13.6
Ear Aspect (Score)	33.3	44.8	78.1	21.9
Grain Moisture (%)	37.5	31.2	68.7	31.3

Total GCA = sum of Male and female GCA sum of squares

Tables 5.10 and 5.11 present GCA effect estimates of the lines as female and male sources, respectively, for yield. Inbreds CML442 (Class 1 = Drought tolerant), CML445 (Class 1), CML488 (Class 1), A7 (Class 2 = Conventional), A8 (Class 2) and B21 (Class 2) had significant positive GCA as both male and female sources for yield across environments. Inbreds CML312 (Class 1), CML444 (Class 1), B16 (Class 3 = GLS tolerant), B17 (Class 3) and B19 (Class 2) had significant positive GCA effects as female sources. Lines A9 (Class 2), A14, A26 (Class 2) and CIM24 (Class 1) had positive GCA effect as male sources. Fifteen hybrids had significant (P<0.05) positive SCA effects for grain yield across environments (Appendix 3).

Table 5.10: Female inbred GCA effects estimate for grain yield across six environments

Inbred	KRC2005	SV2005H	SV2004MS	SV2005LP	SV2004L	SV2004H	Across
CML442	-0.01	-0.01	0.16	0.01	0.62 **	0.02	0.12 *
CML312	1.00 *	0.07	0.11	-0.20	0.97 **	1.70 *	0.61 **
CML445	0.92 *	0.49	0.54	0.39 *	-0.13	0.53	0.46 **
CML395	-0.16	-0.41	0.24	0.04	-0.54 *	-0.17	-0.17 *
CML444	-0.26	-0.15	0.36	0.21	0.19	1.05 *	0.23 **
CML488	0.90 *	0.23	0.02	0.08	0.24	-0.02	0.24 **
A7	0.14	0.89 *	-0.19	0.26	0.04	-0.07	0.18 *
A8	0.29	-0.16	0.07	0.36 *	0.41 *	-0.68	0.05
A9	-0.56	0.00	0.33	0.11	0.02	-0.75	-0.14 *
K64R	-0.96 *	0.17	0.38	-0.24	-0.34	-1.00 *	-0.33 **
B11	-0.71	0.07	-0.56	-0.11	0.41 *	-0.73	-0.27 **
931485	0.54	-0.75 *	0.21	-0.01	-0.31	0.32	0.00
A13	-0.63	-0.23	-1.14 *	-0.72 *	-0.33	-1.30 *	-0.72 **
A14	0.27	0.19	0.71	0.011	-0.94 *	0.08	0.04
A15	-0.58	-0.65 *	0.09	-0.11	0.01	-0.87	-0.35 **
B16	0.22	0.12	0.89 *	0.18	0.37 *	0.79	0.43 **
B17	0.94 *	0.25	0.58	-0.01	-0.39 *	0.37	0.29 **
B18	-1.13 *	0.12	-0.34	-0.46 *	-0.41 *	-038	-0.43 **
B19	1.10 *	0.44	-0.11	0.01	0.16	0.40	0.33 **
B20	0.42	-0.13	-0.79 *	-0.02	-0.28	0.37	-0.07
B21	0.82 *	-0.16	-0.86 *	0.16	0.59 *	-0.52	0.01
B22	-0.83 *	-0.31	-0.26	0.09	-0.46 *	0.42	-0.22 **
B23	-0.16	-0.25	0.36	0.06	-0.01	-0.12	-0.02
B24	-1.46 **	0.20	-0.82 *	-0.12	0.13	0.62	-0.24 **
SED (5%)	0.582	0.421	0.606	0.238	0.273	0.710	0.077

**, * = GCA Significantly different from zero at P≤ 0.01 and P≤ 0.05, respectively; KRC = Kadoma Research Centre in 2004/5; SV2005 = Save Valley in 2004/5; SV2004 = Save Valley in winter 2004; H = high population density and L = Low population density; MS = managed moisture stress

Table 5.11: Male inbred GCA Effect Estimates for Grain Yield across six environments

Inbred	KRC2005H	SV2005H	SV2004MS	SV2005L	SV2004L	SV2004H	Across
CML442	0.62 **	-0.36	-0.16	-0.16	0.37	0.68	0.17 *
CML312	-0.06	0.35	0.24	-0.04	0.17	-0.27	0.07
CML445	-0.53 **	0.50	1.03 *	-0.09	-0.98 **	0.35	0.05
CML395	0.52 **	0.22	-0.42	-0.01	0.04	-0.07	0.05
CML444	1.04 **	-0.41	-0.82	0.28	0.61 **	-0.22	0.08
CML488	0.79 **	0.34	-0.51	-0.12	-0.18	0.53	0.14 *
A7	-0.15	0.09	0.59	0.23	0.49 *	1.47 *	0.45 **
A8	0.32	0.74 *	0.13	0.06	0.87 **	0.62	0.46 **
A9	1.65 **	-0.28	0.09	-0.09	0.11	0.17	0.28 **
A13	-0.86 **	0.09	-0.42	-0.06	-0.33	-0.88	-0.41 **
A14	0.85 **	0.44	0.46	0.39	0.12	0.25	0.42 **
A15	-0.13	0.20	0.18	0.39	0.67 **	-0.87	0.08
B16	-1.25 **	-0.35	0.18	0.14	-0.06	-0.02	-0.22 **
B17	-0.86 **	-0.05	-1.00 *	-0.06	0.09	0.05	-0.30 **
B18	-0.35	0.04	0.090	-0.06	-0.38	0.88	0.04
B19	-0.53 **	0.27	0.240	-0.09	-0.64 **	-0.48	-0.21 **
B20	-0.45 *	-0.53	-1.0 *	-0.41	-1.06 **	-1.00	-0.74 **
B21	0.04	-0.43	0.41	-0.32	0.44 *	-0.67	-0.09
B22	0.12	0.10	0.53	0.13	-0.24	-0.70	-0.01
B23	-0.61 **	-0.48	-0.72	-0.27	-0.21	-0.54	-0.47 **
B24	-0.65 **	-0.13	0.23	-0.21	0.21	-0.18	-0.12
CML489	-0.35	-0.81*	-0.39	-0.66	-0.45 *	-0.65	-0.55 **
A26	0.50 *	0.40	0.24	0.44	-0.21	0.40	0.30 **
CIM24	0.32	0.07	0.78	0.54	0.54 *	1.12 *	0.56 **
SED (5%)	0.619	0.448	0.644	0.253	0.290	0.755	0.065

**, * = GCA Significantly different from zero at P≤ 0.01 and P≤ 0.05, respectively; KRC = Kadoma Research Centre in 2004/5; SV2005 = Save Valley in 2004/5; SV2004 = Save Valley in winter 2004; H = high population density and L = Low population density; MS = managed moisture stress

Table 5.12 presents inbred GCA effects for the number of ears per plant. Inbreds CML442, CML488, A8 and B21 had positive GCA effects as both male and female sources across environments. Inbreds CML312 and A14 had positive GCA as female and male across environments, respectively. Inbreds CML445, B12 and B19 had positive GCA under random stress, whereas B18 and B23 had positive GCA under managed stress.

Tables 5.13 and 5.14 present inbred GCA effects for ASI. Inbred lines CML445, CML444 and A8 had negative GCA as both male and female sources for ASI across environments. The inbreds K64R, CML488 and B19 had negative GCA as female, whereas B22, A26 and CIM24 had negative GCA as male across environments. Lines B17 and B24 had negative GCA under random stress, while B11 had negative GCA as female under well-watered, but low population environment. Inbred A14 had negative GCA under managed moisture stress, but B20 and CML489 had negative GCA under well-watered low population conditions.

Appendices 3 and 4 present GCA estimates of inbred lines for the number of days to reach 50 % anthesis as female and male, respectively. Inbreds CML445, B16 and

B22 had negative GCA effects for anthesis both as male and female sources across environments. The lines A9, B12, A14, A15 and B17 W had negative GCA as female, while CML488, A9, B21 and B19 had negative GCA as male across environments. Line K64R and B24 had negative GCA as female and male, respectively, under random stress, whereas A26 and CML312 had negative GCA under well-watered, but low population environment.

Table 5.12: Inbred GCA Effects Estimate for Number of Ears per Plant across three environments with significant GCA data

Inbred	KRC2005H		SV2004MS		SV2004L		ACROSS	
	Female	Male	Female	Male	Female	Male	Female	Male
CML442	0.00	0.07	0.03	0.03	0.27 **	0.02	0.10 **	0.04 *
CML312	0.17 *	-0.11	-0.09	0.06	0.20 **	-0.06	0.07 **	-0.03
CML445	0.15 *	0.00	-0.07	0.03	-0.03	-0.18 *	0.02	-0.05 **
CML395	-0.01	0.09	0.01	-0.02	-0.13 *	-0.18 *	-0.02	-0.04 *
CML444	-0.06	0.12	0.04	-0.01	-0.03	-0.07	-0.02	0.02
CML488	0.15 *	0.20 *	0.16 *	-0.07	0.32 **	0.14 *	0.18 **	0.09 **
A7	0.00	0.07	0.04	0.03	-0.03	0.14 *	0.00	0.08 **
A8	0.02	0.05	-0.11	-0.16 *	0.20	0.30 **	0.04 *	0.07 **
A9	-0.10	0.20	0.06	-0.01	-0.03	0.00	-0.02	0.07 **
K64R	-0.16 *	---	0.06	---	-0.10	---	-0.06 **	--
RA5414P	-0.11	---	-0.11	---	-0.10	---	-0.10 **	--
B12	0.15 *	---	0.09	---	0.09	---	0.09 **	--
A13	-0.15	-0.13	-0.11	-0.17 *	-0.13 *	-0.01	-0.12 **	-0.11 **
A14	0.07	0.12	0.16 *	0.18 *	-0.13 *	0.07	0.03	0.12 **
A15	-0.05	-0.06	-0.07	-0.01	-0.01	0.09	-0.05 **	0.01
B16	0.07	-0.16 *	0.08	0.02	0.05	0.09	0.04 *	-0.02
B17	0.15	-0.16 *	-0.04	-0.22 **	-0.13 *	-0.01	-0.03	-0.13 **
B18	-0.26 **	-0.16 *	0.08	0.16 *	-0.13 *	-0.15	-0.10 **	-0.05 **
B19	0.20 *	-0.13	0.08	0.08	-0.18 *	-0.11	0.02	-0.06 **
B20	0.10	-0.15 *	-0.16 *	-0.22 **	-0.08	-0.23 **	-0.04 *	-0.20**
B21	0.10	0.05	-0.02	0.13	0.15 *	0.07	0.07 **	0.08 **
B22	-0.06	0.09	0.01	0.06	-0.10	-0.08	-0.03	0.02
B23	-0.06	-0.10	0.18 *	-0.04	0.07	0.04	0.03	-0.03
B24	-0.36 **	-0.11	-0.27 **	0.03	-0.05	-0.06	-0.13 **	-0.05 **
CML489	---	0.02	---	0.06	----	0.02	---	0.03
A26	---	0.09	---	0.04	---	0.02	---	0.05 **
CIM24	---	-0.03	---	0.11	----	0.10	---	0.06 **
SED (5%)	0.103	0.110	0.094	0.100	0.095	0.101	0.075	0.077

**, * = GCA Significantly different from zero at P≤ 0.01 and P≤ 0.05, respectively; KRC = Kadoma Research Centre in 2004/5; SV2004 = Save Valley in winter 2004; H = high population density and L = Low population density; MS = managed moisture stress

Table 5.13: Inbred Female GCA Effects Estimate for Anthesis and Silking Interval across environments.

Inbred	KRC2005H	SV2005HP	SV2004MS	SV2005LP	SV2004LP	SV2004H	Overall
CML442	0.74	-0.10	0.01	-0.27	-0.83	-1.14	-0.27 *
CML312	-0.59	0.90	-0.55	0.23	-0.33	-0.81	-0.19
CML445	-0.59	-0.270	-2.22 **	0.56	-0.83	-0.47	-0.64 **
CML395	0.91	-0.44	0.62	-0.60	0.00	0.36	0.14
CML444	-2.09 **	-1.60 **	-1.89 **	-0.94 *	1.50 **	-1.81	-1.14 **
CML488	-1.26 *	-0.94 *	-0.38	-0.77	-1.17	0.03	-0.75 **
A7	1.58 **	0.56	0.28	-0.27	0.17	0.36	0.45 *
K0315Y	-0.76	-0.27	-0.72	-0.60	0.00	-0.31	-0.44 *
A9	0.74	1.40 **	-1.05 *	2.23 **	1.17	1.36	0.97 **
K64R	-0.59	-0.44	-1.22 *	-0.10	-2.17 **	-1.64	-1.03 **
RA5414P	2.41 **	0.06	0.78	-0.60	-1.67 *	-1.14	-0.03
931485	-0.76	0.56	0.78	0.40	-1.17	-0.47	-0.11
A13	-0.09	0.06	0.78	0.56	0.00	1.86	0.53 **
A14	1.24 *	-0.10	-0.55	-0.44	0.17	2.36	0.45 **
A15	0.58	1.56 **	1.45 **	-0.10	0.00	0.86	0.72 **
B16	-0.26	-0.10	0.78	0.23	1.17	0.19	0.34 *
B17	-1.26 *	0.73	0.78	-0.60	-0.17	-0.64	-0.19
B18	0.91	0.23	0.28	-0.27	0.33	-0.31	0.20
B19	-0.76	-0.60	-1.05	-0.27	0.67	-0.31	-0.39 **
B20	-0.42	-0.60	0.78	-0.44	1.33 *	0.19	0.14
B21	-0.76	-0.10	-0.22	0.56	1.00	0.86	0.22*
B22	0.08	-0.10	1.12 **	0.73	0.17	0.86	0.47 **
B23	0.08	1.23 *	0.45	0.73	-0.33	0.19	0.39 **
B24	0.91	-1.60 **	0.78	0.06	0.67	-0.47	0.06
SED (5%)	0.772	0.655	0.771	0.571	0.875	0.894	0.109

**, * = GCA Significantly different from zero at P≤ 0.01 and P≤ 0.05, respectively; KRC = Kadoma Research Centre in 2004/5; SV2005 = Save Valley in 2004/5; SV2004 = Save Valley in winter 2004; H = high population density and L = Low population density; MS = managed moisture stress

Table 5.14: Inbred Male GCA Effects for Anthesis and Silking Interval

Inbred	KRC2005H	SV2005H	SV2004MS	SV2005L	SV2004L	SV2004H	ACROSS
CML442	-0.42	1.06 *	1.28 *	-0.10	1.00	-0.47	0.39 *
CML312	0.24	0.73	0.28	0.06	0.00	0.36	0.28 *
CML445	-0.42	-0.94	0.28	-0.60	0.33	-0.64	-0.33 *
CML395	0.41	0.23	1.45	0.06	2.17 **	0.86	0.86 **
CML444	-1.09 *	-1.77 **	-1.72 **	-0.44	0.17	-0.97	-0.97 **
CML488	-1.26 *	0.23	-0.22	0.23	0.67	0.86	0.09
A7	1.41 *	-0.44	-0.38	0.56	-1.00	0.19	0.06
K0315Y	-1.43 *	-0.77	-1.55 *	-0.10	-1.67 *	-2.14 **	-1.28 **
A9	-0.42	1.73 **	-0.72	0.06	0.67	-0.47	0.14
A13	-0.09	1.90 **	0.12	0.90 *	0.00	0.53	0.56 **
A14	0.24	-0.10	-1.22 *	0.40	-0.17	-0.31	-0.19
A15	1.41 *	-0.10	-0.38	0.06	1.50 *	1.19	0.61 **
B16	0.24	-0.10	-0.22	0.56	0.50	-0.31	0.11
B17	-0.42	0.06	1.45 *	0.73	0.167	0.86	0.47 **
B18	1.24 *	-0.44	1.12	0.23	-0.17	0.03	0.34 *
B19	0.41	-0.10	0.45	-0.10	-0.17	1.03	0.25
B20	-0.26	-0.44	0.45	-1.27 **	0.00	0.86	-0.11
B21	1.58 *	2.06 **	0.78	1.40 **	0.67	3.19 **	1.61 **
B22	-0.59	1.23 *	-0.72	0.06	-2.33 **	-1.97 *	-0.72 **
B23	0.74	-0.21	0.45	-0.27	-1.33 *	-0.47	-0.18
B24	0.91	-0.77	0.62	-0.10	-1.33 *	-0.81	-0.25
CML489	-0.76	0.44	0.45	-1.27 **	0.83	0.53	0.04
A26	-0.92	-0.27	-0.55	-0.27	-0.67	-0.64	-0.55 **
CIM24	-0.76	-2.27 **	-1.55 *	-0.77	0.167	-1.31	-1.08 **
SED (5%)	0.821	0.697	0.820	0.607	0.930	0.950	0.131

**, * = GCA Significantly different from zero at P≤ 0.01 and P≤ 0.05, respectively; KRC = Kadoma Research Centre in 2004/5; SV2005 = Save Valley in 2004/5; SV2004 = Save Valley in winter 2004; H = high population density and L = Low population density; MS = managed moisture stress

5.4   Discussion

5.4.1 Performance of Hybrids and Inbred Lines

The study revealed significant variability among hybrids and inbred lines for yield and drought stress tolerance and it suggests that hybrids which combine high yield and drought stress tolerance can be identified. Five hybrids among the most resistant had 120% relative yield to the overall mean, which was equivalent to the best commercial standard hybrid (ZS255), which is drought tolerant. While the study confirmed drought tolerance of CIMMYT lines such as CML445, CML395 and CML444 as they were part of the most resistant hybrids. Some conventional inbred lines (B19 and B20) and GLS resistant lines (B17, B16 and A14) were drought tolerant. These conventional inbred lines had high yield both under stress and well-watered conditions, and were also parents of the highest yielding and drought tolerant hybrids. These inbred lines and the specific hybrids with 120% relative yield across drought stress environment can be suggested for use in breeding programmes that emphasise drought stress tolerance. The study also showed that there is still a need to improve levels of drought tolerance in local commercial hybrids as all but one hybrid (ZS255) was classified susceptible. Yield across drought stress environments was significantly correlated with RDT, suggesting that breeding for stress tolerance would not necessarily have negative effects on yield. A positive correlation between IPCA1 and RDT suggested that specific adaptation of the hybrids increased with increasing drought stress tolerance. Conversely, the negative correlation between IPCA1 and yield under well watered conditions, and some random drought stress environments suggested that some hybrids displayed "static" yield stability in these environments. Static stability would not be desired as it implies that farmers would get a yield penalty if high rainfall occurs, but dynamic stability would be obtainable if breeders were to select for prolificacy (EPP) as reported by Zaidi (2002). In this study inbreds displayed variation for EPP for both combining ability and performance per se, indicating availability of local germplasm to select for high number of ears per plant under stress. High levels of heterosis for yield under drought stress that were observed in this study confirmed previous findings (Betran *et al*., 2003), and suggested that these cultivars could also be useful in hybrid-oriented programmes that utilise heterosis.

5.4.2 Gene Action for Grain Yield and its Associated Traits

The study showed highly significant GCA and SCA effects for grain yield, EPP, ASI, flowering dates and other agronomic traits, indicating importance of both additive and non-additive gene action in conditioning these traits. Lager GCA than SCA sum of squares for yield (72%), EPP (77%), ASI (77%), flowering dates (85 to 93%) and other traits (65 to 93%), indicated the predominance of additive over non-additive effects in controlling hybrid performance under moisture stress. These ratios of additive to non-additive effects for grain yield and secondary traits are similar to those (61 to 84%) reported by Betran *et al*. (2003). Grain yield, EPP, ASI and other agronomic traits can be improved by appropriate selection procedures. Significant non-additive portion (±20%) cannot be ignored hence specific hybridisation can also be used especially to enhance grain yield. In a hybrid-oriented programme, additive gene action may be employed to develop stress tolerant inbred lines, by selection and then utilise the non-additive and heterosis by forming hybrids among the identified the inbred lines. Highly significant GCA effects for flowering dates (anthesis and silking) suggests that these traits can be effectively improved through selection, especially in breeding earlier maturing cultivars that can escape drought stress at flowering or late stress that occurs during grain filling. However, observations of highly significant environmental effects and their interactions with GCA and SCA for grain yield, EPP and ASI indicated that in addition to evaluating cultivars under managed drought stress there is also a need to conduct multi-location random stress trials to estimate the G x E component. Crossa *et al*. (1995) reported that crossover interaction resulting in change in cultivar ranking would present problems in varietal selection, as the set of selected hybrids changes in each environment. These results also confirmed previous studies that reported significant interaction of GCA and SCA with environments for yield and its secondary traits (Betran *et al*., 2003).

5.4.3 Combining Ability Estimates

The study confirmed drought tolerance of CIMMYT inbred lines CML442, CML445, CML488 and identified some conventional inbred lines A7, B21 and A8 with positive GCA for grain yield both as male and female sources under drought conditions. These lines can be utilised as source material in breeding for stress tolerance that does not compromise yield. Among these inbred lines CML442, CML488, A8 and B21 also showed positive GCA for number of ears per plant indicating that these lines can be used as donors in breeding for prolificacy in moisture stress environments. Varga *et al*. (2004) reported that selection for prolificacy resulted in

hybrids with greater efficiency than their non-prolific counterparts in using resources under low moisture stress.   Lines that have negative GCA for ASI: CML445, CML444, A8, K64R, CML488 and B19 can be utilised to breed for yield, because ASI was found to be directly correlated with yield under stress.   Inbred lines that had highly negative GCA for DMP: CML445, B22, A9, A14, A15, B17 and B12 can be utilised as sources for earliness where it may be desired to breed for drought escape, especially in situations of late season drought. Predominance of additive gene action suggested that in breeding drought tolerant hybrids both parents should have high levels of grain yield.

5.4.4 Heritability Estimates

Heritability for grain yield decreased with increasing moisture stress and population density across moisture regimes. Heritability was lowest under managed drought stress at flowering (18%) and highest (60%) under well-watered conditions at low population. Under random stress heritability was 24% at 53 000 plants/ha compared to 31% at 22 000 plants/ha. Similarly heritability had a three-fold increase from 21% at 53 000 plants/ha to 60% at 26 000 plants/ha under well-watered conditions. This suggested that in addition to low moisture stress, competition effects at 53000 plants/ha would mask genetic differences among cultivars resulting in reduced selection progress. The best population for selection would be one which is used by farmers in the area. Across environments heritability was 54%, which was comparable to 59% that was reported in tropical populations (Bolanos and Edmeades, 1996). Grain yield heritability observed in this study could be considered high when compared to an average of 18.7% reported by Hallauer and Miranda (1988). Selection would be suggested to improve yield since phenotypic differences would be very high because the ratio of genetic to phenotypic variance would approach unity, especially at low population density under random moisture stress environments.   A similar trend was observed with EPP where heritability was low under high population but increased significantly at low population. Heritability of 42% for EPP under managed stress at flowering was comparable to 45 to 64% reported by Bolanos and Edmeades (1996). Similarly, heritability for ASI was larger (33- 50%) under low moisture stress than under well-watered conditions (19 to 30%) confirming previous studies (Bolanos and Edmeades, 1996). These results indicated that there were small differences between cultivars for ASI under well-watered conditions, but the differences became larger when cultivars were subjected to low moistures stress thereby increasing discrimination between cultivars under drought conditions. It is thus suggested that effective selection of cultivars for ASI should be conducted under

low moisture stress conditions. Heritability of DMS (71%) and DMP (87%), plant and ear heights (33 to 65%) across environments were also within the range of previous findings (Bolanos and Edmeades, 1996; Hallauer and Miranda, 1988). The moderate to high heritability values for these traits suggested that these traits could be effectively improved through selection.

5.4.5 Correlations between Grain Yield and Secondary Traits

 Significant negative correlation between yield and ASI for hybrids (r = -0.34) and inbreds (-0.45) were consistent with previous studies that reported -0.33 to -0.45 under moisture stress (Zaidi *et al*., 2004; Betran *et al*., 2003; Edmeades and Bolanos, 1996).   Strong positive correlation between grain yield and EPP (r = 0.55 to 80) especially under low moisture stress confirmed results reported by previous studies (Betran *et al*., 2003; Bolanos and Edmeades, 1996; Byrne *et al.,* 1995). In contrast with previous findings, there was no significant correlation between grain yield and DMP for hybrids and inbreds under both well-watered or low moisture stress conditions. Betran *et al*. (2003) reported significant correlations between grain yield and DMP (r = -0.16 to -0.45) for hybrids and inbred lines across environments, which would be expected when earlier materials escape drought stress at flowering. The current study observed small, but negative correlation between silking and yield for hybrids (-0.22) and inbreds (-0.36) across environments, suggesting that yield under stress can be improved by selecting for early silk emergence. Zaidi *et al*. (2004) found negative correlations between yield and flowering dates ranging from -0.22 to -0.56, under drought stress, but reported non-significant correlations under optimal conditions. The negative correlations of 0.31 between leaf senescence and grain yield for the inbreds under stress was comparable with previous studies. Banziger *et al* (2000) reported low to medium correlation of yield with leaf rolling and leaf senescence under drought. Ability of tolerant cultivars to capture more solar energy for a longer period during photosynthesis would boost grain filling resulting in higher yield. Correlations between ear aspect scores and yield would be expected, because grain yield is dependent on cob size and grain filling, which are part of the elements of ear aspect scores (CIMMYT, 1985).   Previous studies have not considered this trait, but Zaidi *et al*. (2004) evaluated 100-kernel weight and found a positive relationship with yield. The lack of significant correlation of grain moisture with yield was consistent with previous studies (Betran *et al*., 2003).

5.4.6 Relationship between Environments

Correlation between well-watered and managed drought stress environment at flowering was not significant, indicating that these environments would rank hybrids differently for grain yield. Similarly, there was a weak but positive correlation between well-watered and random drought stress environments, suggesting that selection under optimum conditions may not identify cultivars that are suitable for drought prone areas. Although correlations between random and managed drought stress at flowering were positive (0.23 to 0.36), these were not significant, suggesting that there is need to integrate managed drought stress at flowering with multi-location trials in evaluating cultivars. There were also weak but positive correlations (0.17 to 0.31) among random stress environments, which could be explained by differences in altitude between KRC (1150 mask) and SV (556 m.a.s.l.) and population density effects for two the trials that were established at different populations at Save Valley (SV2005L and SV2005H). Lack of strong positive correlations between managed drought stress at flowering and random drought stress could also be explained by seasonal differences. Managed stress experiments were planted in winter when night temperatures were relatively low, while random stress experiments were conducted during summer and were subjected to high temperatures, suggesting that drought and heat stress effects could be confounded during summer trials. There was a strong positive correlation between random sites and well-watered control planted at 26 000 plants/ha. Probably, higher populations (44 000 to 53 000 plants/ha) in random stress (KRC2005; SV2005) could have accounted for the yield differences. Results from the current study were in agreement with studies by Bruce *et al*. (2002) who reported cultivar x drought interaction resulting in different ranking of hybrids between different water regimes caused by variation in maize susceptibility to drought around flowering. The main impediment of response to selection would be presented by crossover interactions, which leads to re-ranking of cultivars, because it changes composition of the selected and rejected sets across different environments (Crossa *et al*., 1995; Cooper and Delacy, 1990). Future studies should also investigate optimum populations for selecting cultivars in these stress environments. For example, Zaidi (2002) reported that in dry areas of South Africa, farmers' plant at 10 000 plants/ha but cultivars for that environment are selected for prolificacy so that in case of high rainfall the yield potential can be fully exploited.

5.5   Conclusions

The objective of this study was to evaluate: a) grain yield potential; b) levels of drought stress tolerance; c) gene action; d) heritability; e) combining ability; and f) correlations between grain yield and secondary traits under drought stress and well-watered growing conditions. Fifteen experimental hybrids and one standard hybrid (ZS255) displayed high levels of drought tolerance. The following hybrids combined high yield potential (≥120% of overall yield mean) and high tolerance to drought and would be considered for release: 04C2179 (121.6%), CML444/CIM24 (119.6%), A14/B19 (127.5%), CML445/A7 (120.1%) and B17/CML445 (124.8%). Heritability for grain yield decreased with increasing drought stress, from 60% under well-watered conditions to 19% under drought stress at flowering. Heritability for ASI and EPP increased with increasing moisture stress. The study showed highly significant correlation between yield and number of ears per plant and ASI, suggesting that indirect selection for yield can be employed under drought stress. Predominance of additive effects for yield and its associated traits suggested that in forming hybrids both parents should carry high levels of drought tolerance. The following inbred lines that displayed highly positive GCA effects for grain yield under drought stress would be recommended for use as sources for breeding: CML442, CML488, CML312, A14, A8 and B21. The study identified hybrids that combined high yield potential and high tolerance to drought stress relative to the commercial standard hybrid (ZS255), suggesting that drought tolerance was not negatively associated with yield potential in this set of germplasm. It could be concluded that hybrids, which combine high yield potential and high tolerance to drought stress could be developed from these inbred lines.

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Appendices

Appendix 1. Sets of crosses formed in a North Carolina II Mating design (Principal Selection criteria: D = drought tolerance, C = conventional, G = GLS resistance); Conventional means lines were selected on the basis of high yield potential only.

Set 1: Grey Leaf Spot X Drought Tolerant Crosses

♀/♂	CML442 (D)	CML312 (D)	CML445 (D)
B16 (G)			
B17 (G)			
B18 (G)			

Set 2: Conventional X Drought Tolerant Crosses

♀/♂	CML395 (D)	CML444 (D)	CML488 (D)
B19 (C)			
B20 (C)			
B21 (C)			

Set 3: Drought Tolerant X Conventional Crosses

♀/♂	A7 (C)	A8 (C)	A9 (C)
CML442 (D)			
CML312 (D)			
CML445 (D)			

Set 4: Drought Tolerant X Conventional Crosses

♀/♂	CML489 (C)	A26 (C)	CIM24 (D)
CML395 (D)			
CML444 (D)			
CML488 (D)			

Set 5: Conventional X Grey Leaf Spot Resistant Crosses

♀/♂	A13 (G)	A14 (G)	A15 (G)
A7 (C)			
A8 (C)			
A9 (C)			

Set 6: Conventional X Grey Leaf Spot Tolerant Crosses

♀/♂	B16 (G)	B17 (G)	B18 (G)
B22 (C)			
B23 (C)			
B24 (C)			

Set 7: Grey Leaf Spot X Conventional Crosses

♀/♂	B19(C)	B20(C)	B21(C)
A13 (G)			
A14 (G)			
A15 (G)			

Set 8: Conventional X Conventional Crosses

♀/♂	B22(C)	B23(C)	B24(C)
K64R (C)			
B11 (C)			
B12 (C)			

Appendix 2. Drought tolerance, heterosis and yield of hybrids across 4 environments

Hybrid	Drought Tolerance		Grain Yield			
	RDT Value	Rank	Heterosis (%)	% Mean	Rank	t/ha
B23/B16	83.17	1	-14.8	101.2	39	2.7
A13/B21	70.53	2	-22.0	81.8	69	2.2
A8/A13	70.07	4	100.4	89.2	64	2.4
CML444/CIM24	62.28	5	33.0	119.6	12	3.2
A9/A15	60.18	6	162.0	98.3	43	2.7
B11/B24	60.08	7	71.6	76.8	72	2.1
CML395/A26	59.92	8	158.6	97.3	44	2.6
K64R/B24	59.13	9	127.4	88.0	65	2.4
A14/B19	59.02	10	254.7	127.5	4	3.5
CML445/A7	58.69	11	75.8	120.1	10	3.3
A15/B21	57.91	12	146.5	96.2	47	2.6
A15/B19	57.16	13	13.6	101.6	38	2.8
A7/A15	56.48	14	46.6	106.1	33	2.9
B17/CML445	55.76	15	29.0	124.8	5	3.4
CML445/A9	31.53	68	52.4	111.5	26	3.0
CML444/A26	31.06	69	102.0	116.1	14	3.1
CML444/CML489	30.26	70	46.3	66.8	76	1.8
B19/CML488	29.64	71	126.1	124.4	7	3.4
A7/A13	28.94	72	33.5	93.5	51	2.5
B21/CML488	27.70	73	14.2	91.8	55	2.5
B17/CML442	26.85	74	124.3	98.4	42	2.7
B20/CML395	26.69	75	-20.9	93.0	52	2.5
CML312/A7	26.54	76	167.7	100.7	40	2.7
B20/CML444	24.69	77	20.4	94.6	48	2.6
B22/B18	21.80	78	273.6	82.3	68	2.2
B24/B17	18.56	79	63.4	62.4	80	1.7
A13/B20	13.02	80	76.7	75.3	73	2.0
ZS255	55.75	16		120.0	11	3.3
SC633	49.29	29		115.8	16	3.1
R 201	47.89	32		113.9	17	3.1
ZS257	46.90	34		96.3	46	2.6
SC403	46.33	36		103.2	36	2.8
R 215	41.03	45		89.4	62	2.4
SC513	36.96	56		109.2	28	3.0
SC627	31.55	67		92.9	53	2.5
Mean	44.38		104.42	100.0	40.50	2.71
Minimum	13.02		-80.48	62.40	1.00	1.69
Maximum	83.17		515.77	131.11	80.00	3.55

∗ Hybrids are ranked by drought tolerance value; highlighted are high yielding hybrids

Appendix 3: Hybrids with significant positive SCA for Yield across Environments

FEM	MALE	SV2004H	SV2004MS	KRC2005	SV2005LP	SV2005H	SV2004L	Overall
A13	B20	0.9 *	-0.2	2.0 **	0.3	0.5	0.6 **	0.7 **
A15	B21	1.0 *	0.2	0.4	0.3	0.3	0.7 **	0.5 **
A14	B22	0.4	0.6	0.8 *	0.1	0.4	0.4 *	0.5 **
B12	B24	1.2 *	0.0	1.0 *	0.5 *	0.8 *	-0.7 **	0.5 **
K64R	B22	0.8	0.7	1.1 *	0.0	0.4	-0.3	0.5 **
B11	B22	0.9 *	-0.5	0.6	0.5 *	0.6 *	0.5 *	0.4 *
B12	B23	0.5	0.3	0.7	0.0	0.2	0.9 **	0.4 *
B23	B17	0.6	-0.3	0.7	0.1	0.7 *	0.6 **	0.4 *
A15	B19	0.3	0.0	0.9 *	0.4 *	0.4	0.5 *	0.4 *
B24	B18	-0.8	1.7 **	0.4	-0.2	1.1 **	0.2	0.4 *
A14	B21	1.3 *	-0.7	0.8 *	0.4 *	0.0	0.3	0.4 *
B19	CML488	1.2 *	0.6	-0.6	0.2	0.6 *	0.0	0.3 *
B22	B16	0.1	0.2	0.9 *	-0.2	0.8 *	0.0	0.3 *
B18	CML442	0.3	0.2	0.1	0.6 *	0.0	0.3	0.3 *
A7	A13	1.8 **	-0.5	0.3	-0.3	0.1	0.1	0.2 *
SED (5%)		0.66	0.57	0.54	0.22	0.39	0.25	0.30

**, * SCA significantly different from zero at P≤0.01 and 0.05

Appendix 4: Inbred Female GCA Effect Estimate for Number of Days to Anthesis

Inbred	KRC2005H	SV2005H	SV2004MS	SV2005LP	SV2004LP	SV2004H	Overall
CML442	1.09 *	1.26 *	0.62	0.73	2.58 **	2.72 **	1.50 **
CML312	1.09 *	1.09 *	1.95 **	2.23 **	1.58 **	0.06	1.33 **
CML445	-1.24 **	-1.74 **	-0.89	-1.60 **	-0.58	-0.44	-1.08 **
CML395	1.09 *	0.42	2.12 **	1.06 *	1.08 *	2.72 **	1.42 **
CML444	3.26 **	2.26 **	3.12 **	2.23 **	2.58 **	3.22 **	2.78 **
CML488	-0.74	0.42	0.95	0.40	-0.25	0.06	0.14
A7	1.42 **	0.76	0.62	1.40 **	1.08 *	-0.78	0.75 *
A8	0.92 *	0.93	2.28 **	1.40 **	3.25 **	3.56 **	2.06 **
A9	-0.74	-1.58 **	-1.72 *	-2.10 **	-3.58 **	-2.28 **	-2.00 **
K64R	-1.08 *	0.42	-1.05	0.06	-0.25	-1.11	-0.50
B11	1.59 **	0.26	-0.88	0.73	-0.08	-0.78	0.14
B12	-2.08 **	-0.41	-0.38	-1.61 **	0.25	0.89	-0.56 *
A13	-0.58	-0.91 *	-1.72 *	-0.27	1.75 **	-1.28	-0.50
A14	-1.24 **	-1.08 *	-3.38 **	-1.27 **	-3.25 **	-5.94	-2.69 **
A15	-1.91 **	-1.41 **	-0.55	-1.27 **	-1.42 *	-0.78	-1.22 **
B16	-0.74	0.09	-1.38 *	-0.77	-1.42 *	-1.11	-0.89 **
B17	-2.91 **	-1.58 **	0.12	-1.94 **	-1.08 *	-0.94	-1.39 **
B18	1.26 **	0.92 *	0.12	1.73 **	-0.08	0.56	0.75 *
B19	1.59 **	-0.24	1.45 *	0.23	0.58	0.89	0.75 *
B20	-0.08	0.59	-0.38	0.40	-0.08	0.39	0.14
B21	-0.58	-0.58	1.62 *	-1.44 **	-0.25	0.06	-0.19
B22	-0.28	-0.24	-1.72 *	-1.27 *	-0.58	-0.44	-0.75 *
B23	0.26	-0.58	-1.22	-0.27	0.25	-0.44	-0.33
B24	0.59	0.93 *	0.28	1.23 *	1.42 *	1.22	0.94 **
SED (5%)	0.556	0.593	0.983	0.666	0.795	1.044	

**, * = GCA Significantly different from zero at 0.01 and 0.05, respectively.

Appendix 5: Inbred Male GCA Effect Estimate for Number of Days to Anthesis

Inbred	KRC2005H	SV2005H	SV2004MS	SV2005LP	SV2004LP	SV2004HP	Overall
CML442	-0.24	-0.08	0.45	-0.27	0.08	-0.11	-0.03
CML312	-0.74	0.428	0.12	0.56	-1.42 *	-0.61	-0.28
CML445	-1.41 **	-0.91 *	-1.72 *	-1.27 *	-1.25 *	0.78	-0.96 **
CML395	1.26 **	0.09	2.45 **	-0.27	1.58 *	1.06	1.03 **
CML444	1.59 **	1.09 *	1.45 *	1.40 *	0.75	2.39 **	1.44 **
CML488	-1.91 **	-1.41 **	-1.22	-1.94 **	-2.08 **	-2.11 *	-1.78 **
A7	1.09 *	1.42 **	0.78	0.06	1.08	0.22	0.78 *
K0315Y	1.09 *	0.92	2.78 **	2.06 **	4.58 **	3.89 **	2.56 **
A9	-1.24 **	-1.74 **	-1.88 *	-0.77	-2.08 **	-1.78 *	-1.58 **
A13	0.09	0.09	0.12	0.40	0.75	0.72	0.36
A14	0.92 *	0.09	-1.05	0.23	-1.08	-1.44	-0.39
A15	0.59	-0.08	2.12 *	0.06	1.08	1.22	0.83 **
B16	-0.74	-0.58	-1.38	-1.27 *	-1.92 **	-1.11	-1.17 **
B17	-0.41	-0.74	-0.88	-0.94	1.92 **	-0.28	0.22
B18	1.76 **	1.42 **	-0.38	1.90 **	1.08	1.72 *	1.25 **
B19	-0.08	-1.58 **	-1.88 *	-0.77	-2.25 **	-3.28 **	-1.64 **
B20	-0.41	0.42	-1.22	0.73	-1.25 *	-1.44	-0.53
B21	-3.24 **	-2.24 **	-2.55 **	-2.77 **	-2.92 **	-3.28 **	-2.83 **
B22	-0.91 *	-1.08 *	-1.72 *	-1.10 *	-1.42 *	-0.44	-1.11 **
B23	0.59	1.09 *	-0.55	0.73	0.58	-0.61	0.31
B24	-1.24 *	0.26	-0.05	-0.44	0.75	0.06	-0.11
CML489	1.76 **	1.59 **	2.95 **	2.23 **	2.58 **	2.89 **	2.33 **
A26	0.26	-0.41	0.95	-0.27	-1.42 *	1.06	0.03
CIM24	1.59 **	1.92 **	2.28 **	1.73 **	2.25 **	2.06 *	1.97 **
SED (5%)	0.592	0.630	1.046	0.708	0.845	1.110	

**, * = GCA Significantly different from zero at 0.01 and 0.05, respectively.

Chapter 6: Analyses of Grain Yield Stability of Southern African Maize Base Germplasm across Drought and Disease Stress Environments

Abstract

Despite the occurrence of large cultivar x environment interaction that impedes breeding progress under highly variable production environments, there is still limited published information on yield stability of Southern African maize. The objective of this study was to: (a) evaluate the level of yield stability; and (b) determine the relationship between yield stability and yield potential in Southern African maize base germplasm. Twenty-seven inbred lines consisting of six gray leaf spot resistant, six drought-tolerant and 15 conventional lines were mated in eight sets, according to a North Carolina Design II. The resulting 72 experimental hybrids and eight standard hybrids were evaluated for yield stability across 10 drought and disease stress environments, using parametric and nonparametric models. Results showed significant variation among hybrids for yield potential and yield stability. Eighty-five percent of the hybrids showed average stability. Eight percent exhibited below average stability and were adapted to favourable environments. Thus, the hybrids CML395/A26, B17/CML312, B24/B16, B21/CML444 and CML312/A7 would be recommended for release in high yielding environments. Six percent displayed above average stability and were specifically adapted to drought stress environments, hence the hybrids B20/CML488, B11/B24, A13/B21, B22/B18, CML312/A9 and ZS255, would be recommended for deployment in low yielding environments. Parametric models showed a highly significant and positive relationship between yield stability and yield potential, while nonparametric models showed a non-significant relationship between yield potential and yield stability. In addition, the study identified some hybrids which combined high yield potential and high yield stability (B16/CML312, B19/CML395 and B18/CML442). The hybrid B16/CML312, which displayed exceptionally high yield potential (115%) and high yield stability, would be recommended for release in all environments. It can be concluded that high yield potential and high yield stability were not mutually exclusive in this set of germplasm.

Keywords: Yield Stability Analysis, Maize, Cultivar x environment interaction

6.1 Introduction

Cultivar x environment interaction (G X E) has been defined as the differential response of cultivars to environmental changes and results in serious consequences for breeding progress worldwide (Vargas *et al.*, 2001; Min and Saleh, 2003; Tariq *et al.*, 2003; Crossa, 1990). Breeding progress is delayed by G X E interactions, especially when it is associated with changes in cultivar's ranking as manifested by different composition of the selected and the rejected sets across different environments (Crossa *et al.*, 1995; Hohls *et al.*, 1995; Cooper and Delacy, 1990), which complicates selection. In tropical Southern Africa, environmental conditions fluctuate considerably across years and locations as a result of frequent droughts, declining soil fertility and pressure from disease and insect pests, and are further amplified by socio-economic constraints faced by small-scale farmers (Banziger *et al.*, 2004; Richardson, 2003; Rosen and Scott, 1992). Banziger *et al.* (2004) reported that large G X E interactions commonly occurred under drought due to variation in timing and severity of drought stress and their interaction with nutrient deficiencies. Small-scale farmers usually lack the means to modify or condition the production environment due to limited access to technology and inputs, especially fertiliser, irrigation facilities and pesticides.

In the event of a large G X E interaction, a viable approach would be to deploy stable cultivars that have little interaction with environments (Piepho, 1994). According to Tollenaar and Lee (2002), yield stability measures the ability of a cultivar to maintain relative performance across wide environments. An appropriate stable cultivar would be one that is capable of using resources that are available in high yielding environments, while maintaining above average performance in all other environments (Finlay and Wilkinson, 1963). In spite of large G X E problems, cultivars are only tested in a few sites, but are destined for deployment to the whole region due to limited financial resources, especially in national programmes. As a result there is limited information that is published on the levels of grain yield stability in Southern African maize germplasm. It is important, therefore, to evaluate representative germplasm under representative environments to obtain vital information on their adaptability and stability. This information would be used in making breeding decisions about whether to breed for specific or wide adaptation. The objectives of the current study were to: (a) evaluate the level of grain yield stability; and (b) determine the relationship between yield stability and grain yield

potential in a selected, but representative sample of Southern African maize base germplasm across drought and disease stress environments.

6.2   Materials and Methods

6.2.1 Germplasm

Inbred lines used in the study comprised a sample representing the following heterotic groups and their derivatives: i) CIMMYT (International Maize and Wheat Improvement Centre) A and B, and ii) regional: P, K64R, SC, N3, M and I. Features of these germplasm groups have been presented in detail (Mickelson *et al*., 2001; Gevers and Whythe, 1987). Twelve inbred lines comprising six drought-tolerant and six gray leaf spot (GLS) resistant were obtained from CIMMYT-Zimbabwe, and 15 conventionally bred lines were obtained from Seed Co (Table 6.1). These inbreds were specifically adapted to tropical environments in east and Southern Africa. Parent inbred lines were crossed in field nurseries at Muzarabani (±500 m altitude) and Rattray Arnold Research Station (1350 m altitude) in Zimbabwe during winter of 2003 and summer of 2003/4. Inbred lines were crossed in eight sets according to a North Carolina Design II mating scheme (Robinson and Comstock, 1948, 1952). Each inbred line was used once as a female parent in one set and once as a male parent in another set, except the inbreds K64R, B11 and B12, which were used as females only, and CML489, A26 and CIM24, which were used as male parents only (Appendix 1, in sets 8 and 4, respectively). The inbreds CML489, CIM24 and A26 replaced K64R, B11 and B12 as male in set 4, which had failed to provide adequate pollen due to severe attack by maize streak virus. Consequently, eight sets of hybrids comprising nine hybrids each were formed among the 27 inbreds. Commercial hybrids (R201, R215, SC627, ZS257, ZS255, SC403 and SC513, SC633) that are widely grown in Zimbabwe and southern African countries were used as standard check hybrids in the study. The 27 inbred lines, their 72 crosses and 8 standard hybrids effectively constituted the reference population for the study.

6.2.2 Experimental Design

Experiments involving 72 experimental hybrids and eight standard hybrids were established in Zimbabwe (Save Valley, ART, Rattray Arnold, Stapleford and Kadoma) and South Africa (Cedara) during the winter of 2004 and summer of 2004/5 (Table 6.2). The experiments were laid out as an 8 x 10 α-lattice designs. Extra

environments were created at Save Valley by employing different planting densities and watering regimes as suggested by Eberhart and Russell (1966). In terms of the African mega environments, locations represented lowland tropical dry (0 to 1000 masl), mid altitude dry (1000 to 1600 masl), and mid altitude warm and humid environments (1000 to 1600 masl) (Table 6.2). Cultivars were subjected to different levels of disease and drought stress in each environment (Table 6.2).

Table 6.1: Parent Inbred Lines used in a Design II Mating Scheme

Designation	Heterotic Group	*Principal Selection Criteria
CML442	A	Drought Tolerance
CML312	A	Drought Tolerance
CML445	AB	Drought Tolerance
CML395	B	Drought Tolerance
CML444	B	Drought Tolerance
CML488	B	Drought Tolerance
A7	M	Conventional
A8	M	Conventional
A9	P	Conventional
K64R	K	Conventional
B11	K	Conventional
B12	K	Conventional
A13	A	Gray Leaf Spot Resistance
A14	A	Gray Leaf Spot Resistance
A15	N	Gray Leaf Spot Resistance
B16	I	Gray Leaf Spot Resistance
B17	B	Gray Leaf Spot Resistance
B18	B	Gray Leaf Spot Resistance
B19	K	Conventional
B20	KB	Conventional
B21	K	Conventional
B22	S	Conventional
B23	S	Conventional
B24	S	Conventional
CML489	AB	Conventional
A26	I	Conventional
CIM24	A	Drought Tolerance

*Conventional lines were not primarily bred for resistance to stress, but high yield potential.

Table 6.2: Data for the 10 Environments in Zimbabwe and South Africa (Cedara)

Environment	Location	*Season	Latitude	Longitude	Alt. masl	Plants /ha	Major Stress
ART2005	ART	2004/5	17^0 41' S	31^0 04' E	1527	53000	Disease
ST2005	Stapleford	2004/5	17^0 43' S	30^0 54' E	1492	53000	Disease
RA2005	Rattray Arnold	2004/5	17^0 40' S	31^0 13' E	1341	53000	Disease
KRC2005	Kadoma	2004/5	18^0 19' S	29^0 51' E	1149	44000	Drought
CED2005	Cedara	2004/5	29^0 31'S	30^0 17'E	1076	44000	Disease
SV2004HP	Save Valley	2004	20^0 22' S	32^0 17' E	556	53000	None
SV2004LP	Save Valley	2004	20^0 22' S	32^0 17' E	556	26000	None
SV2004MS	Save Valley	2004	20^0 22' S	32^0 17' E	556	53000	Drought
SV2005HP	Save Valley	2004/5	20^0 22' S	32^0 17' E	556	53000	Drought
SV2005LP	Save Valley	2004/5	20^0 22' S	32^0 17' E	556	22000	Drought

*Season: 2004/5 = summer; 2004 = Dry winter (off-season); masl = metres above sea level; ART = Agricultural Research Trust Farm near Harare, Zimbabwe

6.2.3 Management of Experiments

All trials were established with irrigation to ensure adequate plant stand was obtained, but most were later left to rainwater only (Table 6.3). Winter experiments at Save Valley were irrigated throughout the growing cycle, but the managed drought experiment (SV2004MS) received adequate irrigation during the vegetative phase and was subjected to moisture stress at three weeks before anthesis and during the grain filling stage to simulate drought at flowering. Standard cultural practices were followed and experiments were maintained clean of weeds by hand weeding and herbicides. Different amounts of fertiliser were applied in each environment (Table 6.3).

Field data were collected on a whole plot basis following standard procedures used by CIMMYT (CIMMYT, 1985). At harvest all ears were shelled and grain yield (t/ha) was adjusted to 12.5% (Zimbabwe's marketing standards) moisture using the formula:

Grain Yield (t/ha) = [Grain Weight (kg/plot) x 10 x (100-MC)/ (100-12.5)/ (Plot Area)], where MC = Grain Moisture Content.

Table 6.3: Total amount of rainfall and fertiliser applied in each environment

[+]Environment	Rainfall (mm)	Fertiliser (kg/ha)		
		N	P	K
RA2005	826	208	35	21
CED 2005	885	120	33	44
KRC 2005	565	138	64	20
ST2005	814	208	35	21
ART2005	787	250	65	25
SV 2005 H	450	104	18	11
SV 2005 LP	450	104	18	11
SV 2004 HP	Off-season	52	18	11
SV 2004 LP	Off-season	52	18	11
SV2004 MS	Off-season	52	18	11

[+]SV2004 H and SV2004LP = well-watered ± field capacity; SV2004MS drought stressed at flowering

6.2.4 Statistical Analyses

Grain yield stability analyses were performed in Agrobase (Agronomix, 2005) computer package using parametric and non-parametric techniques. Advantages and limitations of each of these approaches have been presented in detail (Crossa, 1990; Lin *et al*., 1986). Although different parameters were used to estimate stability, the

parameters were compared in their ranking of hybrids using correlation analyses. Hybrids that were ranked stable by at least three models were considered stable.

The following methods were used to estimate yield stability of hybrids:

1) AMMI (Additive Main Effects and Multiplicative Interaction) analyses (Crossa *et al.*, 1990; 1991) were performed to predict the hybrid yield means that are adjusted for G x E using the model: $Y_{ij} = \mu + g_i + e_j + \lambda_1 \alpha_{i1} \gamma_{j1} + \lambda_2 \alpha_{i2} \gamma_{j2} + \varepsilon_{ij}$

Where Y_{ij} = yield mean of ith hybrid in j^{th} environment (i =1, 2, 3 …80; j = 1, 2…. 10); μ = grand mean; g_i = main effects of hybrids; e_j = main effects of environments; λ_1 and λ_2 = Eigenvalues for PCA1 and PCA2; $\alpha_{i1}\gamma_{j1}$ = Eigen vectors for PCA1; $\alpha_{i2}\gamma_{j2}$ = Eigenvectors for PCA2; ε_{ij} = random error; PCA1 and PCA2 = principal axes for non-additive variation.

According to Crossa *et al.* (1990; 1991), the hybrids with PCA scores closer to zero could be stable and those with large PCA scores (significantly greater than zero) could be specifically adapted to the environments that have similar large PCA scores with the same sign.

2) Eberhart and Russell (1966) regression analyses were used to determine adaptability and stability of hybrids using the model:

$$Y_{ij} = u + \beta_i I_j + \delta_{ij} + \varepsilon_{ij}$$

Where Y_{ij} = Yield of ith hybrid in the jth environment (i = 1, 2… 80; j = 1, 2 …. 10) μ = Grand yield mean of the ith hybrid over all the 10 environments, β_i = regression coefficient of the ith hybrid. I_j = the environmental index = the mean yield of all hybrids at the jth environment minus the overall trial mean yield; δ_{ij} = deviation of the ith hybrid from the linear regression at the jth environment; ε_{ij} = experimental error.

According to Eberhart and Russell (1966), "hybrids with β = 1.0 have average stability and general adaptation, hybrids with β >1.0 have below average stability and specific adaptation to favourable environments, while those with β < 1.0 have above average stability and are specifically adapted to the low yielding environments. Hybrids with significant deviation from linear regression linearity are not stable."

3) Hybrid superiority of yield across the 10 environments was determined by calculating the cultivar superiority index (Lin and Binns, 1988) using the model: $P_i = \sum(X_{ij} - M_j)^2/2n$ where P_i = superiority index of ith hybrid in the jth environment (i = 1, 2, 3, ….80; j = 1, 2 …10); M = maximum yield for all hybrids in the jth environment; n = number of environments (n = 1 …. 10). The best hybrid would be one with the lowest P_i value.

4) The S^1 and S^2 rank analyses (Huehn, 1990; Nassar and Huhn, 1987; Scapmi *et al.*, 2000) were performed using the following models:

$$S^1_i = \sum_{j<i}|r_{ij} - r_{ij}'|/[n(n-1)/2]; \quad S^2_i = \sum_{j=i}(r_{ij} - r_{ij'})^2/n-1,$$

In this model, S^1_i = the average rank differences for the ith hybrid the across testing environments, r_{ij} = the rank of the ith hybrid in the jth and j' environments, n = number of environments in which hybrids were tested (n = 1 …. 10), S^2_i = variance for the ranks of the ith hybrid across the testing environments. In these statistics, hybrids with small S^1 and S^2 values are regarded as stable (Scapim *et al.*, 2000).

6.3   Results

6.3.1 AMMI Analyses for Hybrid Yield

The AMMI ANOVA analysis revealed significant (P<0.01) differences among hybrids, environments and hybrid x environment interaction effects for grain yield (Table 6.4). The first interaction principal component (IPCA1) and the second (IPCA2) accounted for 72 and 28%, respectively, of the IPCA mean squares. AMMI predicted yield means ranged from 3.9 to 6.8 t/ha across environments (Table 6.5). Rank of hybrids for yield varied in different environments, but the best hybrids dominated the first position in the high yielding environments (Table 6.5 and 6.6).

Table 6.4: ANOVA for the AMMI Analysis of Grain Yield across 10 Environments

Source	d.f.	SS	MS	F-Value	Pr>F
Total	1599	11358.6			
Environments	9	8972.9	996.9	106.8	0.00
Reps (Environments)	10	93.2	9.3		
Cultivar	79	484.5	6.1	3.8	0.00
Cultivar x environment	711	1128.9	1.5	1.8	0.00
1PCA 1	87	390.7	4.4	5.2	0.00
1PCA 2	85	150.3	1.7	2.0	0.00
1PCA Residual	539	587.6	1.0		
Residual	790	678.8	0.8		

Hybrid IPCA1 scores ranged from -1.20 (B11/B24) to 0.99 (CML444/A26) and 11 hybrids had large positive IPCA1 scores (0.5 to 1.0). Twelve hybrids had large positive (IPCA>0.4) and negative IPCA scores (-0.5 to -1.2) (Table 6.5). The remaining 56 hybrids had small IPCA scores, which were not significantly different from zero. Standard hybrids SC627, ZS255, R201 and R215 had large IPCA scores, but only SC627 had a positive IPCA score. Estimates for environmental IPCA scores (Table 6.6) showed that ST2005 (Stapleford) had the largest positive IPCA, while SV2004MS (Save Valley drought stress at flowering) had the largest negative IPCA score. All drought stress environments and the well watered at high (SV2004HP) and low population (SV2004LP) at Save Valley had negative IPCA scores (Table 6.6).

Hybrids CML444/A26 and CML445/A8 were high yielding in favourable and unfavourable environments, respectively, based on AMMI ranking (Table 6.6). Hybrid A14/B19 was among the best only in SV2004MS and the best selections across environments were CML444/A26, B17/CML312, CML445/A7 and B16/CML312 (Table 6.6). There was a positive relationship between IPCA1 scores and yield (Fig 2.). The plot of IPCA1 against IPCA2 scores shows that drought stress and one irrigated but low population environment at Save Valley were grouped together, while the rest were distinct from each other (Fig 3).
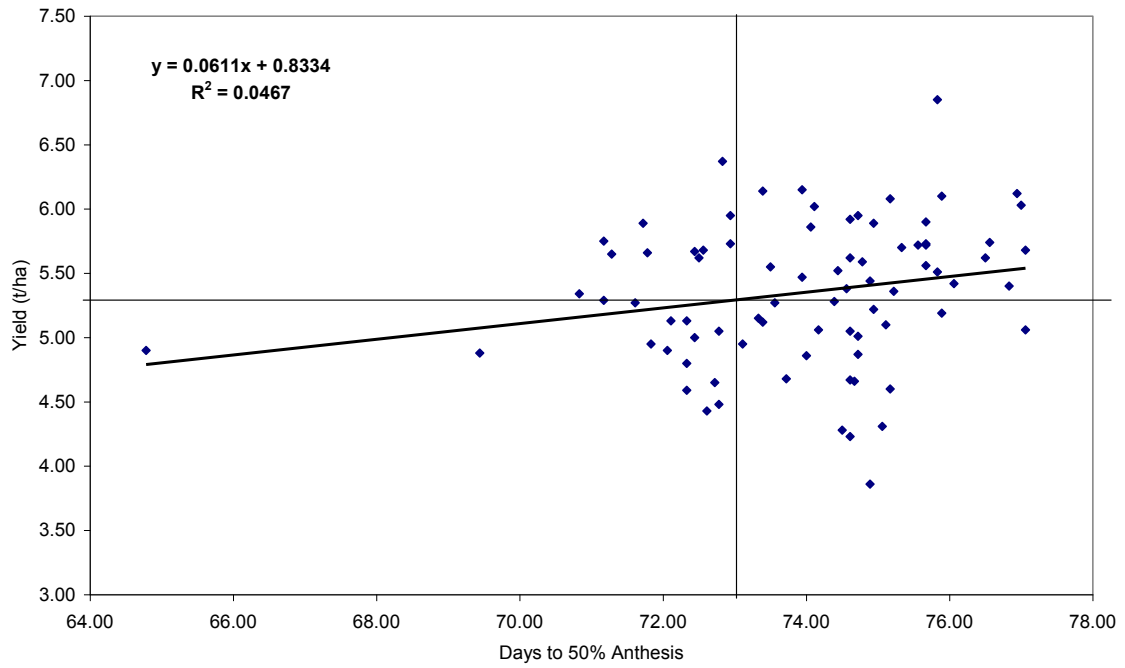
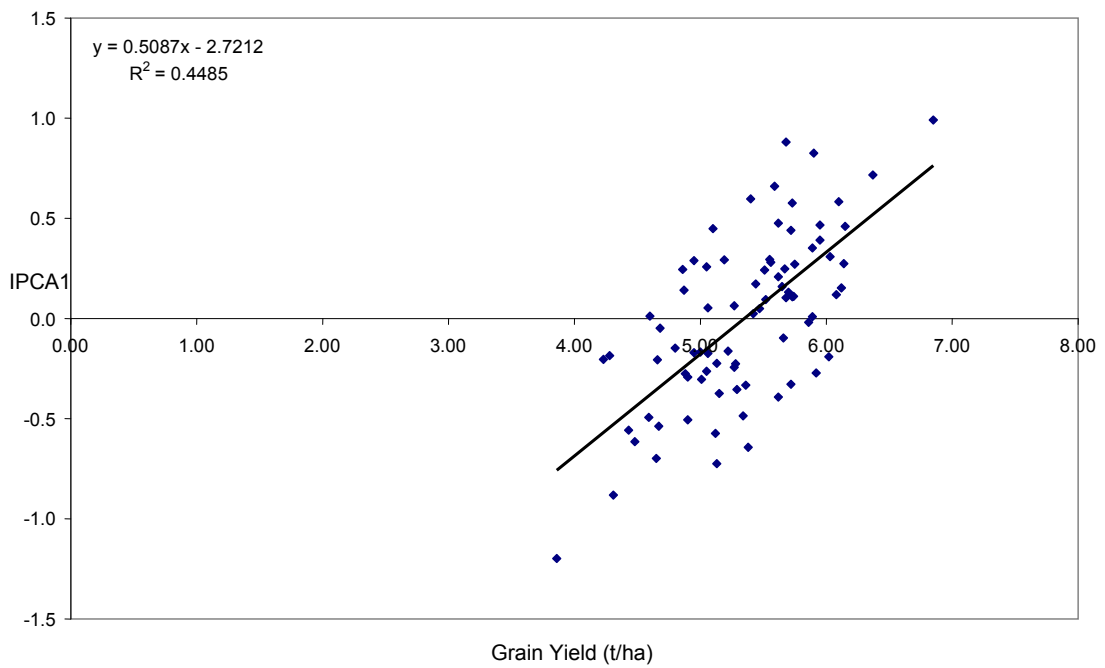Fig. 1: Plot of Yield against days to 50% Anthesis (DMP) of 80 hybrids across 10 Environments

Fig. 2: Plot of IPCA1 against Yield of 80 hybrids across 10 Environments

Table 6.5: AMMI IPCA1 scores and Yield (t/ha) of selected top and bottom yielding hybrids across 10 Environments

NAME	IPCA1	MEAN	*Environment									
			1	2	3	4	5	6	7	8	9	10
Top Yielding hybrids (above mean of 5.3)												
CML444/A26	**1.0**	6.8	10.4	7.8	3.6	11.4	12.5	7.1	6.1	2.9	3.3	3.3
B17/CML312	**0.7**	6.4	9.7	6.6	3.3	10.5	11.6	7.5	5.7	2.7	3.0	3.0
CML445/A7	0.5	6.2	9.1	7.0	3.2	9.9	10.6	6.8	5.8	3.0	3.1	3.0
B19/CML444	**0.6**	6.1	9.2	6.9	3.1	10.0	10.8	6.7	5.6	2.8	3.0	2.9
B16/CML312	0.3	6.1	8.9	6.7	3.3	9.5	10.2	7.1	5.9	3.2	3.3	3.2
CML444/CIM24	0.2	6.1	8.8	6.1	3.4	9.2	10.1	7.8	5.9	3.3	3.3	3.2
CML312/A7	0.1	6.1	8.9	4.1	3.7	9.0	10.6	10.1	5.6	2.9	3.0	2.9
B21/CML395	0.4	6.0	8.8	7.2	3.0	9.6	10.1	6.1	5.7	3.0	3.1	3.0
CML312/A8	0.3	6.0	8.9	5.6	3.3	9.4	10.5	8.2	5.6	2.9	3.0	2.9
B21/CML444	**0.8**	5.9	9.2	7.0	2.7	10.2	11.1	6.1	5.3	2.3	2.6	2.5
CML442/A9	0.5	5.9	9.0	6.3	3.1	9.6	10.6	7.2	5.5	2.7	2.9	2.8
SC633	0.4	5.9	8.7	7.1	3.0	9.4	10.0	6.2	5.6	3.0	3.0	2.9
CML444/CML489	**0.9**	5.7	9.1	5.9	2.6	10.1	11.3	6.8	4.9	1.8	2.2	2.1
B20/CML395	**0.6**	5.7	8.9	6.0	2.8	9.6	10.6	7.0	5.2	2.3	2.5	2.5
SC627	**0.7**	5.6	8.8	5.7	2.6	9.6	10.7	7.0	5.0	2.0	2.3	2.2
CML395/A26	0.5	5.6	8.5	7.3	2.6	9.4	9.8	5.3	5.3	2.6	2.7	2.6
ZS255	-0.5	5.3	7.4	4.9	3.1	7.4	8.0	7.9	5.5	3.3	3.1	2.8
Least yielding hybrids (below mean of 5.3 t/ha)												
A8/A13	0.3	5.2	7.9	6.4	2.3	8.6	9.1	5.5	5.0	2.4	2.4	2.3
SC513	-0.4	5.2	7.2	5.5	2.8	7.4	7.9	6.8	5.3	3.1	2.9	2.7
B24/B17	0.4	5.1	8.1	5.7	2.2	8.8	9.6	6.0	4.7	1.9	2.1	2.0
B24/B16	0.1	5.1	7.6	5.1	2.4	8.0	8.8	6.8	4.9	2.4	2.3	2.2
CML312/A9	**-0.6**	5.1	7.0	5.2	2.9	7.0	7.4	7.1	5.4	3.3	3.0	2.8
A14/B19	**-0.7**	5.1	6.8	6.0	2.9	6.8	6.8	6.3	5.6	3.7	3.3	3.0
B11/B23	0.3	5.0	7.8	5.7	2.2	8.4	9.1	6.0	4.8	2.2	2.2	2.1
B12/B23	-0.3	5.0	7.2	5.2	2.6	7.4	7.9	6.7	5.1	2.9	2.7	2.4
A9/A13	0.3	4.9	7.6	6.8	2.0	8.4	8.7	4.5	4.8	2.2	2.3	2.1
A13/B20	0.2	4.9	7.5	6.1	2.0	8.2	8.7	5.1	4.7	2.1	2.1	2.0
CML488/CML489	0.1	4.9	7.4	6.4	2.0	8.1	8.4	4.8	4.8	2.3	2.3	2.1
SC403	-0.3	4.9	7.0	5.4	2.4	7.3	7.7	6.2	5.0	2.8	2.6	2.3
ZS257	-0.3	4.9	7.1	5.1	2.5	7.3	7.8	6.6	5.0	2.7	2.6	2.3
R201	-0.5	4.9	6.9	4.7	2.6	6.9	7.5	7.1	5.1	3.0	2.7	2.5
K64R/B24	-0.5	4.7	6.5	5.4	2.3	6.7	6.9	5.9	5.0	3.0	2.7	2.4
B22/B16	**-0.7**	4.7	6.4	5.1	2.4	6.4	6.6	6.3	5.1	3.1	2.7	2.4
A14/B20	-0.5	4.6	6.5	5.6	2.2	6.7	6.8	5.5	4.9	2.9	2.6	2.3
B22/B17	**-0.6**	4.5	6.3	4.9	2.2	6.3	6.6	6.2	4.8	2.8	2.5	2.2
R215	**-0.6**	4.4	6.3	4.7	2.1	6.4	6.7	6.2	4.7	2.7	2.4	2.1
B22/B18	**-0.9**	4.3	5.9	3.6	2.3	5.6	6.2	7.3	4.7	2.8	2.4	2.1
B11/B24	**-1.2**	3.9	5.0	4.5	1.9	4.8	4.6	5.6	4.7	3.1	2.5	2.1

* 1 = ART2005; 2 = CED2005; 3 =KRC2005; 4=RA2005; 5 =ST2005; 6= SV2004H; 7 = SV2004HP;

8 = SV2004MS; 9 = SV2005H; 10=SV2005LP; Standard hybrids & significant IPCA scores are in bold.

Table 6.6: AMMI selections for the best four yielding hybrids across 10 environments

| Environment	Mean	IPCA1	Top 4 Hybrids			
			1	2	3	4
ST2005	8.8	2.19	CML444/A26	B17/CML312	CML444/CML489	B21/CML444
RA2005	8.3	1.71	CML444/A26	B17/CML312	B21/CML444	CML444/CML489
ART2005	7.8	1.07	CML444/A26	B17/CML312	B21/CML444	B19/CML444
CED2005	5.9	0.30	CML444/A26	CML395/A26	A7/A15	B21/CML395
KRC2005	2.7	-0.62	CML312/A7	CML444/A26	B19/CML488	CML445/A8
SV2004LP	5.3	-0.68	CML444/A26	B19/CML488	CML445/A8	B16/CML312
SV2005LP	2.6	-0.79	B19/CML488	CML444/A26	CML445/A8	CML488/CIM24
SV2004H	6.5	-0.81	CML312/A7	CML312/A8	CML445/A8	ZS255
SV2005H	2.8	-0.96	B19/CML488	CML445/A8	A7/A14	CML488/CIM24
SV2004MS	2.8	-1.41	A7/A14	A14/B19	B19/CML488	CML445/A8
Overall	5.3		CML444/A26	B17/CML312	CML445/A7	B16/CML312
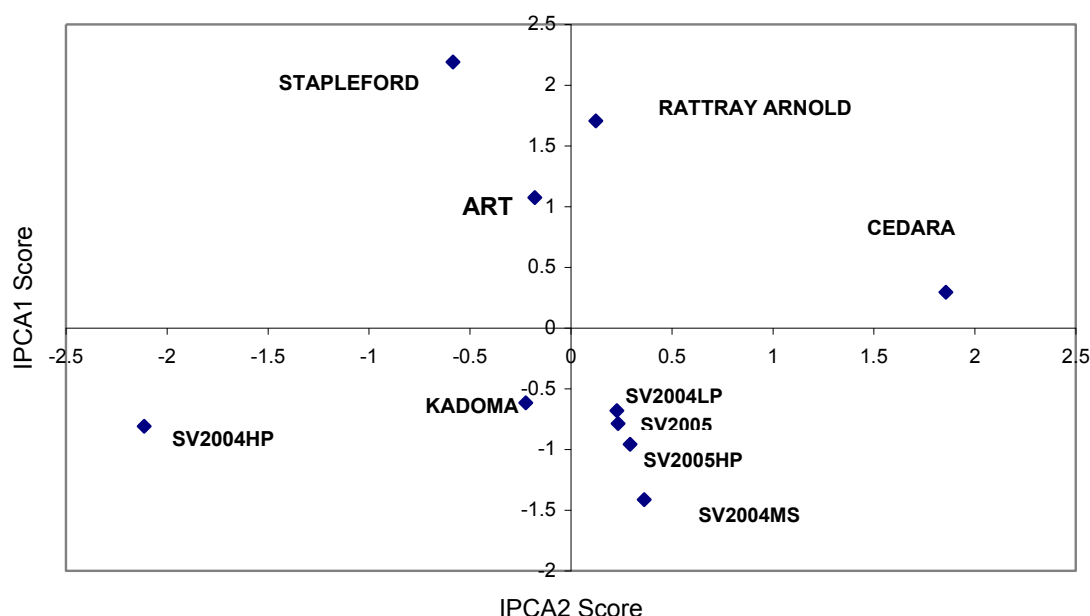
Fig. 3: Plot of IPCA1 scores against IPCA2 scores for 10 environments

6.3.2 Regression Analyses of Hybrid Yield

There were significant differences among hybrids for grain yield due to variation of hybrids, environments and hybrid x environment interaction (Table 6.7). The regression value (β) ranged from 0.48 to 1.45, but only 12 (14%) hybrids had significant deviation from regression linearity of response (Table 6.8). Among them five had β>1.0, six had β <1.0 but one hybrid had β = 1.0. One standard hybrid ZS255 (β = 0.8) showed significant deviation from regression linearity. Two hybrids (CML312/A7 and B21/CML444) with β >1.0 had high relative yield (>110%). The

remaining 68 (86%) hybrids had non-significant deviations from regression linearity of response. Regression coefficients had a positive relationship with grain yield of hybrids (Fig 4).

Table 6.7: ANOVA for the Eberhart–Russell Regression Analysis of Hybrid Grain Yield

Source	df	SS	MS	F-Value	Pr>F
Total	1599	5293.247			
Hybrids	79	242.273	3.067	4.84	0.0000
Env. In hybrids x Env	720	5050.974	7.015		
Env. In linear	1	4486.483			
Hybrids x Env. (Linear)	79	158.560	2.007	3.16	0.0000
Pooled deviation	640	405.931	0.634		
Residual	800	386.058	0.483		

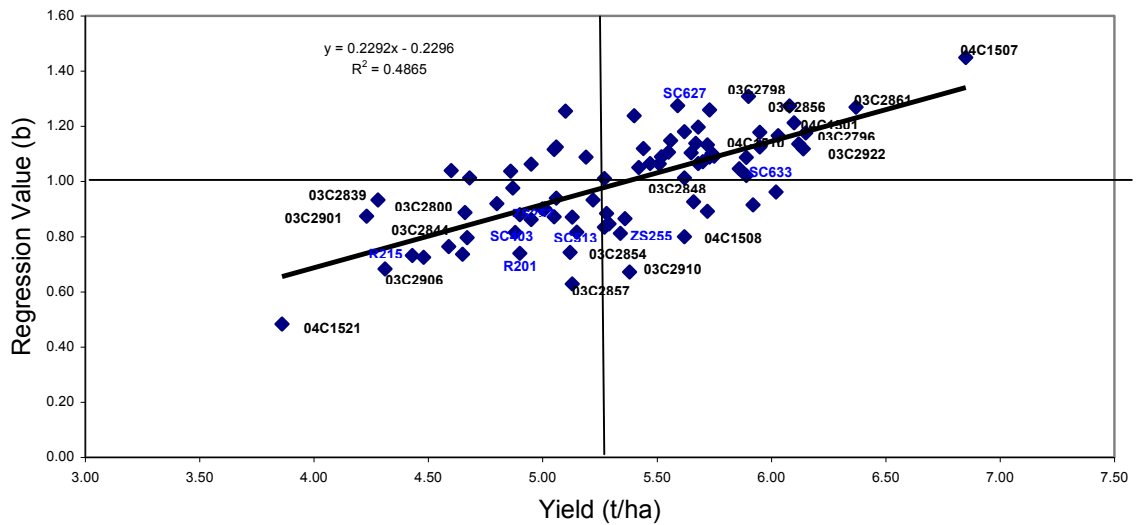Grand Mean = 5.347 R^2 = 0.92   β-value = ± 0.1063   CV = 18.37%

Fig. 4: Plot of Regression Value against Yield of 80 hybrids across 10 environments

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Table 6.8: Grain yield regression and deviation from linearity of response for selected highest yield and lowest yield (<100%) hybrids

Name	Yield		β-value	Deviation*
	t/ha	% Mean		
CML444/A26	6.9	128.5	1.4	0.19
B21/CML444	5.9	110.7	**1.3**	0.77
SC627	5.6	104.9	1.3	0.17
CML312/A7	6.1	114.1	**1.3**	1.67
B17/CML312	6.4	119.5	**1.3**	0.60
B20/CML395	5.7	107.5	1.3	0.34
B24/B17	5.1	95.7	1.3	0.12
CML395/CML489	5.4	101.3	1.2	-0.10
B19/CML444	6.1	114.4	1.2	0.17
CML444/CML489	5.7	106.6	1.2	-0.27
CML395/A26	5.6	105.4	1.2	0.52
CML442/A9	6.0	111.6	1.2	0.11
CML445/A7	6.2	115.4	1.2	0.05
CML312/A8	6.0	113.1	1.2	0.39
B24/B16	5.1	94.9	**1.1**	0.75
B21/CML395	6.0	111.6	1.1	-0.08
B18/CML442	5.4	102.1	1.1	-0.36
B16/CML312	6.1	115.2	1.1	-0.38
SC633	5.9	110.5	1.1	0.17
CML395/CIM24	5.7	106.9	1.1	-0.05
B23/B17	5.5	102.6	1.1	-0.24
04C2182	5.7	106.6	1.1	0.06
B19/CML395	5.5	103.4	1.1	-0.25
A13/B20	4.9	91.2	1.0	0.90
B17/CML445	5.9	110.5	1.0	0.17
A8/A15	5.6	105.4	1.0	0.28
A13/B19	4.7	87.8	1.0	0.22
B21/CML488	5.3	98.9	1.0	-0.06
CML488/CML489	4.9	91.4	1.0	0.16
A13/B21	4.8	90.1	0.9	0.88
ZS257	4.9	91.9	0.9	-0.32
K64R/B23	4.2	79.4	0.9	-0.17
A15/B19	5.1	94.7	0.9	-0.32
B20/CML488	5.1	96.2	0.9	0.50
SC513	5.2	96.6	0.8	-0.27
SC403	4.9	91.6	0.8	-0.28
ZS255	5.3	100.2	**0.8**	1.80
CML488/A26	5.6	105.4	0.8	-0.18
K64R/B24	4.7	87.6	0.8	0.04
A14/B20	4.6	86.1	0.8	-0.10
CML312/A9	5.1	96.1	**0.7**	1.50
R201	4.9	91.9	0.7	0.08
R215	4.4	83.1	0.7	-0.33
B22/B17	4.5	84.1	0.7	-0.21
B22/B18	4.3	80.9	**0.7**	1.01
B11/B24	3.9	72.4	**0.5**	0.74

*Values in bold show significant variation from linearity; β-value = ± 0.1063; standard hybrids are in bold.

6.3.3 Cultivar Superiority Index

Hybrid superiority index ranged between 1.121 (CML444/A26) and 10.235 (B11/B24) (Table 6.9). All the top 20 hybrids and the SC633 had high relative yield (>106%), but the cultivar superiority index had a negative correlation with yield (Table 6.11).

Table 6.9: Cultivar Superiority Index of top and bottom yielding hybrids across environments

♣ Name	Yield		Cultivar Superiority Index
	t/ha	% Mean	
CML444/A26	6.85	128.52	1.12
B17/CML312	6.37	119.51	1.73
B16/CML312	6.14	115.20	1.73
CML444/CIM24	6.12	114.82	1.88
CML445/A7	6.15	115.38	1.88
CML312/A7	6.08	114.07	1.93
B19/CML444	6.10	114.45	2.02
B19/CML488	6.02	112.95	2.05
CML442/A9	5.95	111.63	2.12
B17/CML445	5.89	110.51	2.20
CML445/A8	5.92	111.07	2.24
CML312/A8	6.03	113.13	2.25
B21/CML395	5.95	111.63	2.26
B16/CML442	5.86	109.94	2.37
SC633	5.89	110.51	2.51
SC627	5.59	104.88	2.97
SC513	5.15	96.62	4.21
B12/B22	5.00	93.81	4.52
B20/CML488	5.13	96.25	4.53
CML442/A8	5.06	94.93	4.56
ZS255	5.34	100.19	4.59
A15/B19	5.05	94.75	4.60
ZS257	4.90	91.93	4.74
SC403	4.88	91.56	5.14
CML488/CML489	4.87	91.37	5.16
R201	4.90	91.93	5.20
B18/CML445	4.66	87.43	5.66
A13/B21	4.80	90.06	5.71
B18/CML312	4.60	86.30	5.80
K64R/B24	4.67	87.62	5.91
B22/B16	4.65	87.24	6.12
B22/B17	4.48	84.05	6.53
R215	4.43	83.11	6.60
A15/B20	4.28	80.30	6.90
K64R/B23	4.23	79.36	7.27
B22/B18	4.31	80.86	7.50
B11/B24	3.86	72.42	10.24

♣ Standard hybrids are in bold

6.3.4 Rank Analyses

The S^1 and S^2 values for the hybrids varied significantly from 9.56 (K64R/B23) to 36.22 (ZS255), but only 11 hybrids had significant S^1 values and the remaining 69 hybrids had non-significant S^1 values (Table 6.10). Among hybrids with significant S^1 and S^2 values were standard hybrids: ZS257, R215 and SC513. The second most stable hybrid (B16/CML312) had high relative yield (115%), while two other stable hybrids B19/CML395 and B18/CML442 had average relative yield of 103% and 102%, respectively (Table 6.10). Compared to S^1 analysis, S^2 analysis ranked only six hybrids including one standard hybrid (ZS257) as having significant S^2 values (Table 6.10).

Table 6.10: Hybrids with low and significant χ^2 values for S^1 and S^2 rank analyses

Name	Yield		Rank Analyses Values[♣]	
	(t/ha)	(% Mean)	S^2	S^1
K64R/B23	4.23	79.36	**58.00**	**9.56**
B16/CML312	6.14	115.20	**84.49**	**11.53**
B19/CML395	5.51	103.38	**87.56**	**11.73**
A15/B20	4.28	80.30	**127.01**	**12.11**
B18/CML442	5.44	102.06	**134.40**	**14.49**
ZS257	4.90	91.93	**137.64**	**14.53**
R215	4.43	83.11	201.60	**16.76**
B18/CML312	4.60	86.30	177.05	**16.82**
CML442/A8	5.06	94.93	271.16	**17.56**
A15/B19	5.05	94.75	199.56	**17.56**
SC513	5.15	96.62	206.29	**17.58**

[♣] Significant values and Standard hybrids are in bold

6.3.5 Correlations between Stability Estimates and Grain Yield

There were significant correlations between IPCA scores, regression (b value) and cultivar superiority index, but these were not correlated with non-parametric statistics (Table 6.11).   Regression coefficients had a negative relationship with cultivar superiority index (Fig 5). The IPCA scores had a positive relationship with regression values (Fig 6).   There were significant correlations between S^1, S^2 and regression deviation values in ranking hybrids for yield stability across environments (Table 6.11). Nonparametric stability estimates S^1, S^2 and deviation from regression linearity did not show a significant correlation with grain yield (Table 6.11).

Table 6.11: Correlations between stability statistics in ranking hybrids and hybrid
yield

Statistic	S^1	S^2	IPCA1	IPCA2	β- Value	Deviation	Yield
C S I♣	-0.05	0.02	-0.70 **	0.29	-0.73 **	0.102	-0.967 **
S^1		0.97 **	0.06	-0.11	0.05	0.64 **	0.11
S^2			0.04	-0.13	0.03	0.69 **	0.06
IPCA1				-0.00	0.96 **	-0.04	0.67 **
IPCA2					-0.15	-0.20	-0.28
β- Value						0.04	0.70 **
Deviation							0.02

♣CSI = cultivar superiority index, **, * = Significant at 1 and 5%, respectively.
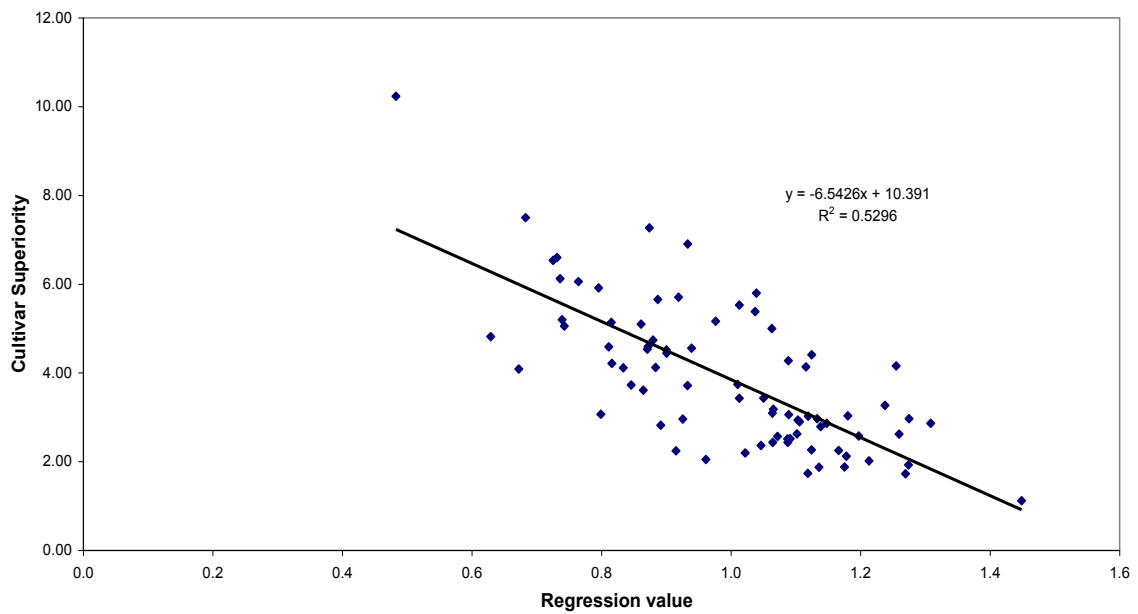
Fig. 5: Plot of Cultivar Superiority index against Regression Value for yield of 80 hybrids
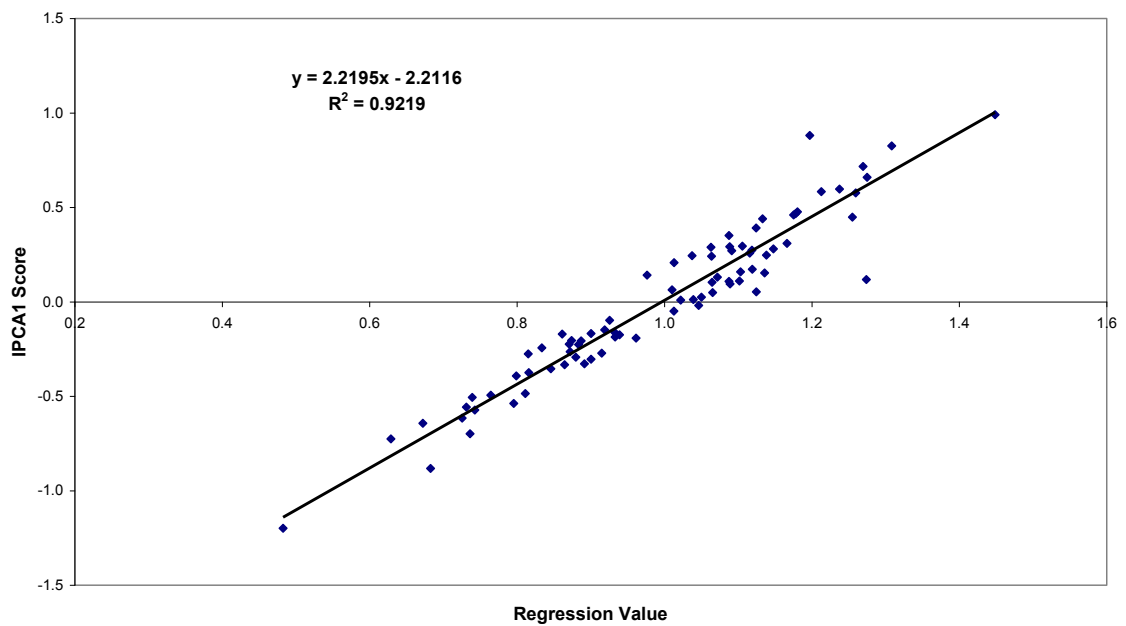
Fig. 6: Plot of IPCA scores against Regression Value for Yield of 80 Hybrids

6.4   Discussion

6.4.1 Relationship between Stability Measurements

Correlations among adaptability or stability statistics provide convincing evidence about stability of the hybrids in these environments. Correlation between regression coefficient and cultivar superiority index of hybrids was significantly negative, indicating that highly responsive hybrids had low superiority indices for grain yield. Correlation between regression (β-value) and IPCA scores was highly significant and positive, indicating that the highly responsive hybrids had positive IPCA scores and would be adapted to high yielding environments. Thus, regression coefficients, IPCA scores and cultivar superiority indices had similar ranking of hybrids for stability. It is suggested that scientists could use any of these approaches to identify hybrids that display dynamic stability or adaptability across environments. In addition, these methods were highly correlated with grain yield; hence they can be employed as effective tools in identifying hybrids, which combine high yield potential and high yield stability across stress environments. On the contrary, there was no significant correlation between parametric methods and nonparametric methods, indicating that in general these methods would not have similar ranking of hybrids. Also the non-parametric estimates had significant and positive correlation between them, indicating that these stability measures were similar in ranking hybrids for stability, which was consistent with previous findings in Brazilian maize (Scapim *et al.*, 2000). Again scientists would use any of these models to evaluate hybrids for stability. In the current study, hybrids were considered stable if they appeared stable in more than three stability analyses.

6.4.2 Grain Yield Stability

Significant variation of hybrids for grain yield, which was explained by genetic variation among hybrids, environmental main effects and the hybrid x environment interactions in this study is supported by abundant literature involving both tropical African and temperate maize germplasm (Banziger *et al.*, 2004; Lee *et al.*, 2003; Min and Saleh, 2003; Tariq *et al.*, 2003; Worku *et al.*, 2001). In the current study, there were experimental hybrids that had high relative yield ranging from 110 to 128.5% (Table 6.9). These hybrids showed a 10% yield advantage over the best standard hybrid (SC633), and had the lowest cultivar superiority values; hence they were the

best performing hybrids. These hybrids would be recommended for further testing and subsequent release or for use as breeding sources. The highest yielding hybrid CML444/A26 showed average stability and was well adapted to all environments. In fact the hybrid CML444/A26 was among the best four hybrids in 7 of the 10 environments (Table 6.6). The hybrid also displayed the lowest cultivar superiority index, indicating that it was the most superior hybrid across environments. Despite having a β-value of 1.4, the hybrid did not show any significant deviation from linear regression, indicating that it was stable and responsive to environmental change. Theoretically, this hybrid could be recommended for deployment in all environments. However, this hybrid was not identified as stable by the nonparametric methods and had a large IPCA score; hence it was not considered among the most stable hybrids. In addition, the hybrid CML444/A26 had above mean number of days to anthesis, suggesting that it would be late maturing. As a result it might not be practical to deploy the hybrid in tropical lowland dry environments where the growing season is ultra short, but it is a very promising candidate for release in the high potential long season environments. The data did not show a significant correlation between yield and number of days to 50% anthesis (Fig. 1), suggesting that in general anthesis period could not explain differences in yields among hybrids. High yield potential for some hybrids could be due to heterosis effects, which was expected, because the hybrids were formed between lines that were heterotic or from divergent backgrounds.

Highly significant environmental effects could be explained by different drought and disease stress conditions and altitude. Clearly, hybrid yield was lowest in drought stress environments at very low altitude within the tropical lowlands at Save Valley, while it was highest at high input and high altitude environments such as Stapleford and ART. Cedara had significantly lower yields due to high disease pressure than ART and Stapleford. There were high levels of gray leaf spot (GLS) and phaeosphaeria leaf spot (PLS) diseases at Cedara. High levels of hybrid x GLS and hybrid x PLS disease interactions were expected at Cedara, whereas they were absent at Stapleford where there was neither GLS nor PLS. This is supported by the long distance between Cedara, Stapleford and ART on the plot of IPCA1 against IPCA2 scores for the environments (Fig. 3). In contrast, there was a shorter distance between drought stress environments, suggesting that these environments had similar interaction with hybrids. Differences between hybrids could be explained by hybrid x drought interactions at lowland tropical dry environments at Save Valley (SV2005L and SV2005HP). Kadoma, which is at mid altitude, was some distance

apart from the other random drought environments, suggesting different interaction with hybrids, which could be explained by the higher altitude. The high altitude environments generally experience lower temperatures and the growing cycle for maize was longer than in the lowland environments. Long growing period has been reported to be associated with high yielding potential (Pingali and Pandey, 2001; Beck *et al.*, 1990). Thus, in addition to hybrid x stress interaction effects, hybrid x altitude interaction effects could also be inferred from this study.

The plot of IPCA1 and IPCA2 scores (Fig. 3) also indicated that artificially managed drought stress at flowering formed a distinct environment (SV2004MS). Although a short distance away from others, this environment was not grouped together with the random drought stress environments, suggesting that it had a different interaction with hybrids. Differences between hybrids in this environment could be partly caused by interaction of hybrids with drought at flowering and grain filling stages. Bruce *et al.* (2002) reported cultivar x drought interaction resulting in different ranking of hybrids between different water regimes caused by variation in maize susceptibility to drought around flowering.

The plot of IPCA1 and IPCA2 scores (Fig. 3) showed that the optimum moisture environment (SV2004HP) had distinctive interaction with hybrids, which was different from the other environments. It has a long distance from the entire drought and disease stress environments (ART, Cedara and Rattray Arnold) and the highest yield potential (Stapleford). Clearly, it is indicated that selection under well-watered or optimum moisture conditions at 53 000 plants/ha might not identify hybrids that are adapted to drought or moisture stress. In addition, this was a winter environment and was at lower altitude than other high yield potential environments. Low night temperatures, which lengthen the growing cycle, were observed by large number of days to anthesis suggesting that low night temperatures could have confounded drought.

The clustering of environments could be explained by the differences in altitude, hybrid interaction with drought stress, hybrid interaction with altitude and hybrid interaction with plant population density. All environments at Save Valley had negative IPCA scores, which can be attributed to their low altitude. The well-watered environments were placed in separate quadrants, which could be explained by the different population densities of 53000 (SV2004H) and 26000 (SV2004L). Thus, these environments had different ranking of hybrids due to the hybrid x population

density interaction effects. Although it is difficult to explain why the well-watered low population (26000) environment (SV2004H) was closer to the random drought and low population (22000) environment (SV2005), it can be speculated that these had similar hybrid x population density interaction effects. Perhaps, these environments had similar ranking of hybrids suggesting non-crossover G x E interaction.

In this study, hybrids were identified with different levels of stability and with average and specific adaptation to high yielding and drought stress environments. Twelve hybrids that showed significant deviation from linearity of response could be regarded as unstable across these environments. According to Eberhart and Russell (1966) hybrids with significant deviations from regression linearity of response are not stable and their performance would not be predictable. Five of these 12 hybrids (CML395/A26, B17/CML312, B24/B16, B21/CML444 and CML312/A7) had high regression coefficients ($\beta > 1.0$); hence they displayed below average stability and could be classified as specifically adapted to high yielding environments (Fig. 4). These hybrids would be recommended for deployment in high yielding environments. Six hybrids (B20/CML488, B11/B24, A13/B21, B22/B18, CML312/A9 and ZS255) had β-value < 1.0, indicating above average stability and specific adaptation to drought stress environments (Fig. 4). Apparently, one hybrid (A13/B20) had a β-value of 1.0, indicating average stability, but was not stable. Thus, despite having a β-value of 1.0, the hybrid A13/B20 had a significant (P<0.01) deviation from the linear regression, indicating that its performance would not be predictable across environments. The study showed 68 of the 80 hybrids that had no significant deviation from the regression linearity of response, indicating that 85% of these hybrids had average stability in these environments. This could be explained by the fact that experimental hybrids were crosses among drought tolerant and gray leaf spot disease (GLS) resistant inbred lines. Some of the parent-inbred lines were principally selected for drought and GLS resistance, and drought and GLS were experienced in four and three of the ten environments, respectively. Moreover, conventional lines that were used to form hybrids were elite and had been extensively tested through multilocation trials, suggesting that they had been indirectly selected for yield stability and tolerance to drought and disease in random environments.

The following hybrids were identified as stable by nonparametric estimates based on S^1 and S^2 hybrid rank values (from most stable to the least stable): K64R/B23, B16/CML312, B19/CML395, A15/B20, B18/CML442, ZS257, R215, B18/CML312,

CML442/A8, A15/B19 and SC513. These hybrids also displayed non-significant deviation from regression linearity of response and had low IPCA scores (i.e., close to zero); hence they were considered stable by at least three models. All the hybrids except three (B16/CML312, B19/CML395 and B18/CML442) with significant stability as determined by rank analyses were relatively low yielding (<100%), suggesting that they were poorly adapted to all environments. In addition, some standard three-way cross hybrids (R215, SC513 and ZS257) were ranked among the most stable, suggesting that stability could have been conferred by heterogeneity. Eberhart and Russell (1966) reported that use of genetic mixtures rather than homogeneous cultivars reduced G X E interaction. This suggested that more heterozygous and heterogeneous cultivars were less affected by environmental differences due to population buffering. More recently, Lee *et al*. (2003) reported that more homogeneous inbreds and F$_1$ hybrids had larger G X E than heterogeneous double-cross and three-way cross hybrids. In this study, there were many single crosses, which were more stable than the standard (commercial) three-way cross hybrids. Eberhart and Russell (1966) reported that it was also possible that some single crosses had as much or even more stability than most stable three-way and double-cross hybrids.

Despite having relatively low yield (<100%), four of the most stable hybrids contained the conventional inbred K64R or its derivatives, which are extensively used in Southern Africa (Gevers and Whythe, 1987). These conventional lines could have accumulated stability through extensive testing in environments that included random drought and disease stress. Lee *et al*. (2003) reported that stability was heritable, predictable and mostly controlled by additive gene action implying that high yield stability could be accumulated by recurrent selection for mean performance across multiple environments. On the contrary the highest yielding, but unstable (β>1.0) hybrids and specifically adapted to high yielding environments had the following inbred parents: CML444, CML312 and CML395, which were principally bred for drought tolerance. These inbred lines are late in flowering and maturing, which explains their high yield potential (Pingali and Pandey, 2001; Beck *et al*., 1990). It is suggested that drought tolerance in these materials might not be usable in drought-prone lowland dry environments, where the growing season is ultra short. There is need to develop some earlier versions of these lines that have adaptation to drought stress environments within the lowlands. This study suggests that new germplasm combining drought tolerance and earliness can be obtained from crosses between the late maturing CIMMYT inbred lines and K64R derivatives. These can be used as

source populations for pedigree breeding. Some of these CIMMYT lines displayed high grain yield potential in combination with K64R derivatives; hence these crosses can form the basis for pedigree selection.

6.4.3 Correlations between Grain Yield Potential and Yield Stability

A highly significant (P<0.01) and positive correlation (r = 0.7) between yield potential and regression coefficients (Fig. 4) indicated that there was a positive relationship between yield potential and stability in this set of germplasm. Results confirmed a previous study by Worku *et al.* (2001) who reported positive correlation (r = 0.537) between yield potential and regression coefficients in East African maize cultivars. Nonetheless, these results were different from the significant and negative relationship between yield potential and regression coefficients, which was reported in temperate maize (Tollenaar and Lee, 2002; Jansen and Cavalieri, 1983). The differences of results may be explained by the different sets of germplasm and the different test environments. Results from different regression analyses may not be comparable because the environmental index against which hybrid yields are regressed is dependent on the particular set of hybrids (Lin and Binns, 1986; Crossa, 1990). Non-parametric analyses in this study showed no significant relationship between yield potential and yield stability. However, both parametric and nonparametric methods identified hybrids which combined high yield potential and high yield stability indicating that high yield potential and high yield stability were not mutually exclusive. In other words there was no negative association between high yield potential and high yield stability, which has important implications for breeding. Further evidence is provided by the hybrids B16/CML312, B19/CML395 and B18/CML442, which combined high yield potential (>100%) and high yield stability, which would be recommended for release across stress prone environments. These hybrids can also be used as sources in breeding new lines for yield stability. The hybrid B16/CML312, showed exceptional stability in combination with high yield potential. Apart from recommendation for release, this hybrid can be used as an elite breeding source for new stable lines. Although not tested in this study, previous studies by Lee *et al*. (2003) reported that stability was heritable and largely controlled by additive gene action. Thus, selection procedures can be used to develop inbred lines with high yield stability.

6.5 Conclusion

The objective of this study was to: (a) evaluate the level of yield stability; and (b) determine the relationship between yield stability and grain yield potential in a representative sample of Southern African maize base germplasm. Results showed that 85% of the 80 hybrids had average stability across the 10 environments. Eight percent displayed below average stability and were specifically adapted to high yielding environments. The hybrids CML395/A26, B17/CML312, B24/B16, B21/CML444 and CML312/A7 with high yield potential would be recommended for release in high yielding environments. Six percent exhibited above average stability and were specifically adapted to drought stress environments. The hybrids B20/CML488, B11/B24, A13/B21, B22/B18, CML312/A9 and ZS255, would be recommended for deployment only in low yielding environments to which they were specifically adapted. Parametric models showed a highly significant and positive relationship between yield stability and yield potential, while nonparametric models showed no significant relationship between yield potential and yield stability. In addition, the study identified some hybrids that displayed both high yield potential and high yield stability (B16/CML312, B19/CML395 and B18/CML442). The hybrid B16/CML312, which displayed high yield potential and high yield stability would be recommended for release in all environments. It can be concluded that high yield potential and high yield stability were not mutually exclusive in this set of germplasm, indicating that hybrids, which combine high, yield potential and high yield stability would be obtainable.

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7.1   Introduction

The purpose of this chapter is to close the thesis by reviewing and concluding the completed research, and drawing out some of its implications for breeding.   The following research hypotheses were tested across environments in Zimbabwe and South Africa:

a) Small-scale farmers in Southern Africa recognise the "key" production constraints peculiar to their area and have specific preferences for stress tolerant maize cultivars,

b) There is adequate genetic variation for grain yield and its associated traits, which is highly heritable and can be exploited in a breeding programme to generate drought tolerant materials,

c) There is sufficient genetic variation and high levels of resistance to GLS and PLS, which is highly heritable and can be exploited in a breeding programme to generate disease resistant materials, and

d) There is a significant positive relationship between stress tolerance; grain yield potential and yield stability in Southern African adapted maize base germplasm.

7.2   Findings of the Study and Their Implications

Very little research has been published about grain yield potential, resistance to GLS and PLS, and yield stability in Southern Africa maize germplasm. The study, therefore, reviewed work done mainly with temperate maize in temperate environments, but comparison with the Southern African situation was made wherever possible. A huge yield gap between yield potential and farmers' yields was shown in both temperate and Southern African environments. Stress factors were used to explain the yield gap. Significantly high yield potential in temperate maize was accumulated through breeding for stress tolerance, especially at high plant density. Yield stability was achieved through testing in many environments with low precision, but the testing environments were mostly representative of the farmer's situation. The lesson to be learnt for Southern Africa would be to breed for major biotic and abiotic stress resistance in order to increase productivity. The experimental cultivars should then be tested under the conditions that best represent the production environments of small-scale farmers in marginal areas. These farmers produce maize under complex low input systems, because they have limited or no

access to inputs that can be used to modify their production environment. Cultivars that are adapted to high yielding environments on research stations would fail to adapt to the production environment in marginal areas, which is quite different from the research stations. In the current study, it was shown that there was no significant correlation between hybrid ranking in the optimum and moisture stress conditions. Thus, drought tolerant cultivars may not be found by selecting under optimum conditions. The optimum conditions do not exist in marginal areas; hence selection for cultivars to be deployed in marginal areas should be conducted under stressful conditions that best represent the farmers' production environments.

A household survey and participatory rural appraisal (PRA) in the marginal eastern belt of Zimbabwe indicated that farmers recognised the production constraints peculiar to their areas. Production constraints differed significantly between districts due to the different ecologies, which influenced preferences for stress tolerant cultivars. Farmers in areas with potential for producing surplus grain perceived weevil damage to stored grain to be an important constraint. This means that in areas with potential for producing surplus grain, the breeding emphasis should include weevil control. Future research should study the feasibility of screening and breeding for grain weevil resistance, if cultivars were to be deployed in areas where small-scale farmers are expected to produce surplus grain that would be stored on farm. These farmers might not have proper storage facilities for their grain.

The PRA study showed that farmers in the relatively more productive district (Mutasa) perceived low soil fertility as a major production constraint ahead of drought. Thus, farmers in this area would prefer cultivars with tolerance to low soil fertility or with high nitrogen use efficiency. Problems of leaching would be expected to be high in these areas. Thus, in addition to drought or in the absence of drought, low N is one of the major limiting factors. Pingali and Pandey (2001) reported that progress has been made in developing maize cultivars that can efficiently utilise available soil nutrients, especially nitrogen and convert it to grain. The germplasm with low N use efficiency should be obtained and crossed with adapted maize for deployment in wet marginal areas. Although, materials in the current study were not evaluated for low N tolerance, Edmeades *et al*. (1997) reported that many cultivars selected for drought tolerance also had high yield under low N conditions. Breeding for drought tolerance might allow spillover benefits to low N environments. Banziger *et al*. (2002) reported that decreased ear abortion and increased assimilate supply during grain filling of maize selected for tolerance to mid season drought also

provided tolerance to N stress and therefore, may contribute to increased yield and yield stability. There is a complex interaction between available N and drought, such that under drought hybrids are also exposed to low N, because moisture is required for the uptake of nutrients. Nitrogen is taken up dissolved in water and during drought less N is available to the plant leading to apparent N deficiency. Thus cultivars that perform well under low moisture stress are mostly to be equally efficient at utilising the little N available. As a result cultivars that are tolerant to drought may also have some degree of tolerance to low N stress. The hybrids that were found to be drought tolerant in the current study would be recommended for screening under low N to confirm if they have some tolerance to low N stress.

Farmers' preference for ultra early cultivars (< 90 days to harvest in these areas) would not be met by using the set of germplasm in this study. The frequency distribution of germplasm evaluated in the current study was skewed towards late maturing period. Although some hybrids showed exceptional drought tolerance combined with high yield, they were late in flowering, suggesting that they would not fit into the short seasons in drought prone areas. Thus, drought tolerance in late inbreds such as CML444, CML395 and CML488 would not be usable in lowland tropical dry areas, which have short growing seasons of about 90 days. It is thus suggested that these materials should be crossed to early lines to generate breeding populations for selection under drought stress at flowering in lowland tropical environments. Another suggestion by Pingali and Pandey (2001) is to cross late-maturing tropical lines with ultra-early maturing temperate lines. The current study indicates that selection for earliness would be effective. This is because heritability was high for both days to 50% anthesis and silking. In addition, both grain yield and flowering traits were highly influenced by additive gene action. Foreign, but ultra-early materials can be crossed with the highly adapted "K64R" group and CIMMYT sources and then select for high yield, earliness and drought tolerance at flowering.

Small-scale farmers in the marginal eastern-belt preferred hybrids of the 1970's to the new hybrids, made available after 2000. This preference was supported by the observation that the old hybrid "R201", which was highly preferred by farmers, showed a yield advantage of 10% (relative yield = 113%, Chapter 5, Table 5) over the widely grown new hybrids across drought stress environments. This might suggest that little progress has been made in breeding for drought tolerance or acceptable cultivars during the past 35 years. Since the 1970's breeders in Zimbabwe have concentrated on breeding for high yield potential areas. Breeding

goals would have been focused on the requirements of large-scale commercial farmers in high potential environments. The strong correlation between high yield and a long growing cycle might have also resulted in the bias towards the late maturing hybrids in breeding programmes. Beck *et al*. (1990) reported that early materials tend to have lower yield potential than the late materials. The yield "penalty", which is realised with early cultivars, when rainfall levels rise above average, might discourage breeders from selecting for earliness (Banziger *et al*., 2000). Published research indicated that cultivars combining high yield potential and earliness could be obtained by selecting for late leaf senescence. Thus, the high yield potential in temperate maize is partly explained by selecting for late leaf senescence or the "stay green trait" (Duvick, 1997; Tollenaar and Wu, 1999). Breeding for delayed leaf senescence in ultra early hybrids would enhance grain yield through increased leaf area duration, especially during favourable seasons. In addition, breeding for prolificacy would enhance yield, which enables the flexible cultivars to produce an extra ear during favourable seasons. Results from the current study showed that both low leaf senescence score and high number of ears per plant were significantly correlated with high grain yield under drought stress.

In designing cultivars for deployment in marginal areas, as shown by the case of the marginal eastern-belt of Zimbabwe, farmers' perceptions and preferences should be part of the essential elements. According to farmers in the Save Valley area, the best drought tolerance should involve a recovery mechanism to reduce losses that are common during the mid-season drought. Farmers mentioned that such a mechanism was available in sorghum cultivars grown in the area. It is thus suggested that appropriate technology such as molecular technologies that were long ago suggested for use in studying the sorghum resistance should be employed in studying drought tolerance of sorghum land-races in this area. There is a possibility of identifying genes that can be transferred from sorghum into the drought tolerant maize hybrids identified in the study. Such a programme would constitute appropriate biotechnology for small-scale farmers in the dry marginal areas. In the mean time, ways to allocate a certain percentage of the production area to sorghum and pearl-millet in addition to maize for food security need to be recommended where possible through research projects and/or extension. Nevertheless, the switch from maize to sorghum promotion would not be a viable alternative as these farmers showed that they had great interest in maize despite its lack of relative drought tolerance.

The design elements for the farmers' stress tolerant "ideotype" cultivar should include breeding for semi-dent to flint grain texture, which was highly preferred. The other traits preferred by farmers in marginal areas could be bred by crossing the promising drought tolerant materials with local landraces to obtain certain subjective requirements (which would be difficult to measure) such as what farmers referred to as "good taste". The study showed that farmers recognised shortcomings of their cultivars but would hold on to them because of some of these subjective requirements, which breeders may not be able to identify on their own. In contrast breeders have a strong focus on broad adaptation and set their goals towards meeting requirements of large-scale commercial farmers, which may be misdirected in a region where small-scale farmers operating with limited resources dominate the production of maize. A shift in focus towards breeding goals that include the farmers' requirements for stress tolerant cultivars in marginal areas is therefore suggested.

Another finding from the study was that breeding for high resistance to GLS from this regional set of germplasm was highly feasible. Many hybrids and inbreds displayed high levels of resistance to GLS, presenting an opportunity to select for resistance. The inbred lines A13, A15, B18 and B19 would be recommended for use as breeding sources for GLS. High levels of resistance in standard hybrids released during the 1990's and the 2000's in this study also showed that breeders have made more progress in breeding for GLS than drought stress tolerance. These hybrids showed remarkably high resistance to GLS compared to the old hybrids that were released before the GLS epidemic (before 1988). Gray leaf spot disease is more prevalent in high yield potential areas, which attracts the attention of private breeding companies. Secondly, high progress in breeding for GLS can be explained by the large additive gene effects and high heritability estimates. On the contrary it is more difficult to breed for drought tolerance. Thus, new hybrids did not show any yield advantage under drought. Breeders might be discouraged from breeding for drought tolerance due to low heritability of yield under drought stress. Clearly, this study showed that heritability for yield under drought was low and the absence of significant hybrid rank correlations between optimum and stress environments indicated that breeders would not make progress in identifying drought tolerance when they select under optimum conditions. The high yielding potential or humid environments, which attract breeders, would be ideal for identifying disease resistant cultivars, but not drought tolerant cultivars. The study also indicated that breeding for GLS-resistance would not be very complicated because single cross hybrids would be resistant when at least one of the parent inbred lines is resistant to GLS. This suggested that

screening inbreds for resistance *per se* would identify resistance sources that can be used in combination with complementary lines that are selected based on a different criteria. Breeders could choose the second line for use in a single cross hybrid based on resistance to some other important stress or high grain yield potential.

Although PLS may still be regarded as a minor disease, a review of the literature suggested that the PLS epidemic might follow a similar trend to GLS in the USA, which appeared in 1925 but only became an economic disease in the 1970's (Huff *et al*., 1988). The devastating effects of the PLS disease that have already been reported in Brazil support the view that it has the potential of becoming a major disease and threatening regional food security. Observation of an interaction between GLS and PLS on some cultivars indicated that a combination of these diseases poses a challenge to food security. Additionally, there is limited knowledge of the epidemiology of the disease suggesting that resources should be directed towards studying its epidemiology, which would provide additional knowledge in breeding for resistance to PLS in regional maize. There is also limited information or statistics on the incidence and losses that can be incurred when susceptible cultivars are grown in Southern Africa. Therefore, it is recommended that formal research should be conducted to determine the incidence of PLS and the grain yield losses associated with the disease across the subcontinent.

The inbred lines used in the study were not selected for PLS, but substantial resistance was observed in parent inbred lines and experimental hybrids. This indicated that breeders do not have to look beyond the region in search of resistance in order to develop resistant cultivars. Resistance that is obtained in this regional set of germplasm would be most useful, because these lines are already adapted. For example, germplasm obtained from outside the region might not have resistance to maize streak virus, which is peculiar to Africa. This study identified lines that contributed exceptionally high resistance to PLS (B23, B17, B12 and CML444) and would be recommended for use as breeding sources. Since resistance was highly heritable and controlled by genes acting in an additive manner, adequate resistance to PLS can be accumulated though simple selection.

The breeding approach for PLS resistance would be different from that of GLS. Thus, unlike GLS resistance, the study showed that high resistance to PLS would be obtained in hybrids when both parents carry the resistance. Resistance to PLS was inherited predominantly in an additive manner. The non-additive portion of the model

was not significant in the current study. Also Pegoraro *et al*. (2001) reported that resistance to PLS involved two major genes that behaved mainly in an additive fashion. Carson (2005) reported that three to four genes were involved and resistance was predominantly conditioned by additive gene action. This finding has some implications for hybrid oriented breeding programmes. The implication is that resistance to PLS should be found in inbred lines from at least two heterotic groups, because hybrids would be formed between complementary inbred lines (i.e. lines from different genetic backgrounds). The general observation in Zimbabwe is that lines from the "P" heterotic group were generally susceptible to PLS, while those from the "K" had some resistance. Therefore it may be difficult to develop PLS resistant hybrids between inbred lines from the "P" and "K" heterotic groups. Hybrids that are based on these heterotic groups are common in South Africa and Zimbabwe (Olver, 1998; Cowie, 1998; Gevers and Whythe, 1987). The study suggested that PLS resistance has to be improved in the "P" heterotic groups given its regional significance. A similar situation has been reported by Carson (2001) in the USA where inbreds derived from B73 were generally susceptible, while those from the complementary Mo17 derivatives were resistant to PLS. According to Olver (1998) the "P", "K" and B73-type germplasm have been widely used to constitute early hybrids for deployment in marginal areas. The stress tolerant hybrids of the 1970's, namely R200, R201 and R215, were based on inbred lines from the "K" and "P" heterotic groups. Generally, it is recommended that regional lines from the major heterotic groups should be screened for resistance to PLS.

Simultaneous selection for PLS and GLS is suggested, because these diseases usually occur together. New pedigrees can be created by crossing GLS and PLS resistant-lines that were identified in the study. Pedigree procedures can be used in breeding for resistance. In addition, the study showed clearly that resistance of hybrids to PLS and GLS would be predictable as revealed by the high regression values for hybrid means on the mid parent values.

Results also showed large differences between male and female mean squares for GLS and PLS, leading to speculation on the possible role of maternal effects in influencing resistance, which would affect breeding progress. The non-genetic maternal effects, which are not heritable, would impede selection progress. Previous studies did not investigate the role of maternal effects in conditioning resistance. Most of these previous studies reported the predominance of additive over the non-additive effects. If present, maternal effects would cause an upward bias for additive

effects, which has serious implications for the conclusions drawn in the previous studies. The upward bias of additive variance by maternal effects also has serious consequences for estimating heritability, which is a ratio of additive variance to total phenotypic variance. In this study, heritability estimates were not biased by maternal effects because the male variance component was used to estimate heritability. However, caution had to be exercised in interpreting results, because the models used in this study did not make a direct test for the presence of reciprocal effects. Thus, in the current study, maternal effects could only be estimated by comparing the male and female GCA components from the analysis of the design II mating. It is, therefore, recommended that future studies should employ models that incorporate reciprocal effects in order to confirm this "speculative" role of maternal effects in influencing resistance to PLS and GLS. If the role of maternal effects is proven, then breeders should make the resistant inbred line the female parent in designing single cross hybrids.

Another important finding was the positive and significant hybrid rank correlations between environments for both GLS and PLS, which suggested that non-crossover hybrid x environment interaction was involved as opposed to crossover interactions. Non-crossover G X E effects would indicate that hybrid ranks for resistance would not change in different environments reflecting only differences in the magnitude of resistance to levels of the disease pressure in each environment. In this situation, high costs that are incurred in multilocation testing can be avoided, by screening germplasm at one site. Disease "hot spot" sites such as Cedara can be utilised in screening for disease resistance. Compared to RARS and ART in Zimbabwe, Cedara in South Africa, showed significantly higher discrimination of hybrids for resistance. There was no hybrid, which was rated "immune" to GLS at Cedara, in spite of the fact that some hybrids had been rated as "immune" at RARS and ART. Hot spot screening eliminates false escapes that would reduce efficiency or impede selection progress. Any hybrid that is rated resistant at Cedara would be most likely to be resistant at any other site in the region. As a result, inbred lines and hybrids which showed high resistance to PLS and GLS in this study can be recommended for use as resistance sources anywhere in the region, where the diseases present problems.

There was a significantly high positive relationship between grain yield and yield stability, indicating that breeding for high yield stability would not necessarily result in low grain yield potential. Drought stress tolerance index for the hybrids showed a positive relationship with stability across drought stress environments, indicating that

breeding for drought stress tolerance in this set of germplasm would enhance stability of yield. Although the study showed no significant relationship between grain yield and resistance to GLS or PLS, it has to be acknowledged that the effects of GLS and PLS on yield could not be separated as the diseases occurred together. There was an interaction between GLS and PLS on some cultivars; hence there were some confounding effects. In any case, the relationship between grain yield potential and GLS or PLS severity would depend on the time the diseases occur. The diseases have to occur before or during the early grain filling stage to have a significant impact on grain yield. Literature review showed that a negative relationship would be obtained if the GLS occurs early and the incidence is moderate to severe. In the study, the diseases occurred at 50% silking but their incidence intensified during the late grain-filling period, which may explain the lack of a significant relationship between yield and disease severity. However, the study identified hybrids that showed high relative yield advantage under disease and drought stress, indicating that hybrids that combine high yield potential and high resistance to disease or drought stress would be obtainable. In addition, some hybrids combined stress tolerance with high yield potential and high yield stability, supporting the view that stress tolerance, high yield potential and yield stability are not necessarily mutually exclusive.

The study identified experimental hybrids with potential for release. The hybrids A13/B20, A13/B19 and A15/B21, which combined high grain yield (relative yield = 117%) with resistance to both GLS and PLS would be recommended for release in areas with a growing period of up to 150 days, where both GLS and PLS are a problem. The hybrids A9/A13 and B24/B18, which combined high relative yield (117%) with high resistance to GLS, would be recommended for use in areas where GLS is the major biotic stress. In areas, where PLS is more important than GLS, the following hybrids, which combine high relative yield (≥121%) and resistance to PLS would be recommended for release: CML444/A26 (relative yield = 141.9%), B20/CML488 (138%), B19/CML488 (124%) and B21/CML395 (121%). In drought-prone areas, the following hybrids, which combined high drought stress tolerance and high yield potential, would be recommended: 04C2179 (relative yield = 121.6%), CML444/CIM24 (119.6%), A14/B19 (127.5%), CML445/A7 (120.1%) and B17/CML445 (124.8%). These hybrids would best fit in the mid altitude dry areas, such as Kadoma area in Zimbabwe. The hybrids still need to be improved by reducing their maturity period so that they would adaptable to the ultra-short seasons in tropical lowland dry areas such as Save Valley.

In the current study, the hybrid B16/CML312 showed exceptional high stability across drought and high yielding environments. This hybrid displayed a combination of high yield potential (relative yield = 115%) and high stability. The hybrid would be recommended for deployment across disease and drought stress environments with a growing period of up to 150 days. Due to its high stability it has the least chance of disappointing the farmers because farmers would not get a yield "penalty" during the favourable seasons. Apart from releasing it to farmers in marginal areas, the hybrid would be suggested for use as a source of stability in pedigree breeding. Although not tested in the study, literature review showed that stability was highly heritable and was conditioned predominantly by additive gene action; hence it can be improved by selection. Thus, pedigree selection can be used to develop lines with high yield potential and yield stability from this hybrid. As a single cross hybrid, there is also an opportunity to use the hybrid as female in combination with a complementary early inbred line to produce an ultra-early three-way cross hybrid. Alternatively, the hybrid can be crossed to a complementary ultra early single cross and produce a stable, but early maturing double cross hybrid for deployment in marginal areas.

7.3   Conclusion and the Way Forward

Using the case of the marginal eastern-belt of Zimbabwe, the study showed that during the past 35 years maize breeding has not made impact in delivering the farmers' preferred drought stress tolerant cultivars in Zimbabwe. Farmers still preferred the old hybrids of the 1970's, which showed a 10% yield advantage over the new hybrids (of the 1990's and 2000's) that were recommended for their area. Farmers showed their preference for semi-dent and flint grain texture, and cultivars displaying drought stress recovery mechanism comparable to sorghum. Future studies should look at the drought stress mechanism that is expressed by local sorghum races, and investigate the possibility of transferring resistant genes to maize using appropriate technology such as molecular techniques.

The findings from the study suggested that the participatory rural appraisal (PRA) studies, which determine the requirements of the smallholder farmers, who dominate production of the maize crop, should be part of the essential elements of plant breeding programmes in Southern Africa.

A large number of hybrids and inbreds displayed high levels of resistance to GLS and PLS, presenting a huge opportunity to select for resistance. Inheritance of GLS

resistance was to a greater extent conditioned by additive effects and was highly heritable. Single cross hybrids showed high levels of resistance when at least one parent was resistant, suggesting that the second parent in a cross could be identified by some other criteria.

Although PLS is still regarded as a minor disease, the indicated that it has the potential to become a major disease, with serious implications for food security. Additionally, there is limited knowledge of the epidemiology of the disease indicating that resources should be directed towards studying the epidemiology and breeding for resistance to PLS in regional maize. Resistance of PLS was highly heritable and was inherited in a strictly additive manner. Results showed that single cross hybrids resistant to PLS would be obtained when both parents carry the resistance. Future studies should incorporate reciprocal effects to investigate speculation for the possible role of maternal effects in influencing resistance for GLS and PLS.

The study identified hybrids with high yield advantage over standard hybrids under disease and drought stress conditions. These hybrids would be recommended for release and for use as sources of drought and disease resistance in breeding programmes. In sum, the study indicated that farmers' preferences for maize cultivars and traits would be greatly influenced by the major prevailing constraints in their area. It also identified adequate genetic variation for GLS and PLS resistance, drought stress tolerance, high yield potential and high yield stability in Southern African maize base germplam, without negative associations among them, suggesting that cultivars combining high yield potential, high stress tolerance and yield stability would be obtainable.

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