Breeding Investigations for Resistance to Phaeosphaeria Leaf Spot (PLS) and other Important Foliar Diseases and a Study of Yield Stability in African Maize Germplasm

by

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Thesis Abstract

Maize (*Zea mays* L.) yields in the smallholder (SH) farming sector in Southern Africa have remained low, despite the availability of many improved varieties. Among the major constraints contributing to low yields and threatening food security in the region are diseases which include grey leaf spot (GLS), common rust, northern corn leaf blight (NLB) and Phaeosphaeria leaf spot (PLS). These diseases are highly unpredictable in their occurrence every season, making them difficult to control. In addition, the majority of SH farmers cannot afford to control the diseases due to limited access to chemicals. Therefore, maize cultivars with high levels of disease resistance and tolerance to abiotic stresses would provide a long-term solution to addressing the problem of low yields, especially in the smallholder-farming sector. The objectives of this study were therefore to: i) establish farmers' perceptions on diseases, key limiting production constraints and preferred traits of maize cultivars, ii) screen germplasm adapted to tropical environments for resistance to PLS, iii) determine gene action for resistance to PLS and GLS, iv) estimate combining ability effects for resistance to PLS, GLS, NLB and common rust diseases, and v) determine grain yield stability of F_1 hybrids derived from crosses among selected tropical advanced maize inbred lines. These studies were conducted from 2006/7 to 2008/9 seasons at various sites in South Africa, Zimbabwe, Zambia and Uganda.

Structured surveys and participatory rural appraisal (PRA) conducted in Obonjaneni, Busingatha and Okhombe villages of Amazizi district in the Northern Drakensberg established maize as the principal crop grown in the area. All the farmers who participated grew the local variety (landrace) they called *Natal-8- row* or *IsiZulu*. The adoption of hybrids and improved open pollinated varieties (OPVs) was low. Farmers preferred the local variety ahead of hybrids and improved OPVs mainly for its taste, tolerance to abiotic stresses and yield stability. Characteristics of maize varieties preferred by the farmers included: inexpensive seed, high yield, early maturity and low input costs. Pests/diseases and drought were not ranked highly, as farmers planted early to escape diseases and drought. Abiotic stresses were amongst the top four constraints faced by the farmers. The local varieties exhibited high yield potential and genetic variability for disease resistance.

Evaluation of maize germplasm adapted to tropical and subtropical environments of Africa for PLS resistance indicated significant ($P \leq 0.05$) variation among the inbreds,

populations and hybrids. In general, 63% of the inbreds/populations were resistant to PLS. Regionally important inbred lines; SC and N3 and CIMMYT's most successful lines such as CML395, CML444, CML202, CML312, and CML488 were resistant to PLS. Fifty-four percent of the single-cross experimental hybrids were also resistant to PLS. Correlation coefficients for area under disease progress curve (AUDPC) values for disease severity with PLS final disease severity scores were significant ($P<0.001$) and positive, implying that ranking of the genotypes for AUDPC and final PLS disease severity score was by and large similar.

Forty five F_1 hybrids generated by crossing ten advanced maize inbred lines in a half diallel mating scheme were evaluated in two to six environments to determine combining ability, gene action and heterosis estimates for grain yield and resistance to PLS, GLS, NLB and common rust diseases. Highly significant ($P\leq0.001$) general combining ability (GCA) and specific combining ability (SCA) effects were observed for PLS, GLS, NLB, common rust, grain yield and other agronomic traits. The GCA effects were more important than SCA effects, indicating the predominance of additive over non-additive gene action for all the traits studied in these inbred lines. The inbred lines with good GCA for PLS resistance were: A1220-4, N3, A16, MP18 and CML488, and for GLS resistance were A1220-4, CZL00009, CZL00001, CML205 and CML443. Lines A16 and CML443 had good GCA for NLB and common rust resistance, lines A1220-4, N3, CML205, A16, and CML443 contributed towards high yield. Lines A1220-4 and A16 were late maturing, whereas CZL00009 displayed early maturity. High mid-parent and better-parent heterosis for high grain yield and resistance to all the diseases were observed.

Generation mean analysis was used to determine the inheritance of PLS and GLS resistance in populations involving six tropical advanced maize inbred lines. Reciprocal crosses and backcross progenies were generated among inbreds A1220-4, A15, B17 (resistant, R), CML445 (moderately resistant, MR), CML441 and CZL00001 (susceptible, S) for PLS inheritance, and among inbreds A1220-4, A15, CML441 (resistant, R), and N3 and B17 (susceptible, S), for GLS inheritance. Results indicated highly significant additive effects ($P<0.001$) for PLS and GLS resistance, with dominance effects accounting for ≤11% of the variation in all the crosses for PLS and only A15 x B17 cross for GLS. Epistasis and cytoplasmic gene effects in favour of PLS resistance in F_1 crosses when the more susceptible parent was used as female were significant. For GLS resistance, epistasis was observed only in CML441 x N3 and A1220-4 x B17 crosses, while no cytoplasmic gene effects were detected. Resistance for PLS was medium to highly heritable and conditioned by less than four genes which exhibited incomplete

dominance. In general resistance to GLS was controlled by two to three genes exhibiting zero to partial dominance and was moderate to highly heritable.

Stability analysis of the hybrids was done over 11 environments using the additive main effects and multiplicative interaction (AMMI) and the genotype and genotype by environment (GGE) biplot analyses. Both AMMI and GGE biplot analyses selected hybrids H21 (CZL00009 x A16), H14 (A1220-4 x A16), S63 (SeedCo hybrid check), N72 (MP72/N3) and H26 (CZL00001 x A16) as stable and high yielding. Hybrids H1 (CML445 x A1220-4), H44 (CZL00009 x CML443) and H18 (CZL00009 x CZL00001) were identified by both methods as unstable but high yielding. AMMI and GGE biplot analyses identified ZAM08, C108, RA09 and C09 as the most representative environments which were high yielding and relatively stable.

In general, the study has revealed that based on the farmers ranking of the constraints in their area, breeding opportunities do exist for incorporating tolerance to both biotic and abiotic stresses in their varieties. It also identified maize lines resistant to the main foliar diseases, with good combining ability and heterosis for resistance and high grain yield. Hybrids with wide adaptation and high yields across environments were also observed. The experimental hybrids that exhibited high levels of resistance can be recommended for further testing and release. On the whole, highly significant additive effects and moderate to high heritability estimates observed for all the diseases and grain yield implied progress would be made through selection, although significant epistasis and dominance could slow progress. Dominance effects towards resistance and high yield could be exploited in developing single cross maize hybrids among these inbreds when only one parent is resistant.

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Declaration

I, Julia Sibiya, declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. The thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
   a) Their words have been re-written but the general information attributed to them has been referenced.
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Signed:

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

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Professor Pangirayi Tongoona (Supervisor)

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Dr John Derera (Co-Supervisor)

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Dedication

This thesis is dedicated to the people closest to me who have always believed in me; my husband (Job), my children (Joel and Jaimie), my mother (Maina Bonga) and my late father (Mike Bonga).

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Introduction to Thesis

1 Background

Maize (*Zea mays* L.), is the most widely grown food crop in sub-Saharan Africa (SSA) and is produced on approximately 22 million hectares of land, which is about 15.7% of the land area grown to maize globally (Pingali and Pandey, 2001). The total annual maize production in SSA is estimated at approximately 34.424 million tonnes (Aquino *et al*., 2001). Statistics have also shown that out of the 23 countries with the highest per capita consumption of maize as food in the world, 16 are in sub-Saharan Africa. Maize contributes 50% of calories in Southern Africa, 30% in East Africa and ±15% in West and Central Africa (Banziger and Diallo, 2002). However, despite the large scale production of maize in SSA, maize yields in the region have remained low, presenting a big challenge for researchers (Mashingaidze and Mataruka, 1992).

The major constraints contributing to these low yields include biotic stresses (weeds, insect pests and diseases), abiotic stresses (inadequate rainfall, low soil fertility), poor field management and lack of resources (Tattersfield, 1982; Mashingaidze and Mataruka, 1992; FAO and CIMMYT, 1997; Vivek *et al*., 2001). The diseases which are endemic to most SSA maize production areas include maize streak virus (MSV), grey leaf spot (GLS, *Cercospora zeae-maydis* Tehon & Daniels), rust *(Puccinia sorghi* Schwein. and *P. polysora* Underw.), northern corn leaf blight (NLB, *Exserohilum turcicum* Pass. Leornard & Snuggs), ear rots (*Fusarium* and *Diplodia*), head smuts (*Sphacelotheca reliana*) and Phaeosphaeria leaf spot (PLS, *Phaeosphaeria maydis*) (Bonga and Cole, 1997; Vivek *et al*., 2001). These diseases are often difficult to control since their occurrence year after year is less predictable because of their high dependence on weather. As a result, in favourable seasons with high rainfall, diseases also become more prevalent and damaging. The majority of small-scale farmers cannot afford, in most cases, to control the diseases due to limited access to pesticides. Therefore, the development of maize cultivars with enhanced levels of disease resistance and high abiotic stress tolerance will be sustainable and effective for increased maize yields, especially in the smallholder-farming sector.

Although it has not been listed among the dominant diseases, PLS disease has been increasingly observed in various African countries. Currently, there is no literature

available to show the accurate distribution of the disease in Africa, but through *personal communication*[1] (George Bigirwa and Joe DeVries) and personal observations in Zambia, PLS has been reported in southern, eastern and central African countries. Reports of high PLS incidences have come mainly from South Africa, Zimbabwe and Kenya and the disease has great potential to threaten regional food security. Grain yield losses due to PLS are still to be quantified in the region (Vivek *et al*., 2001), but substantial losses ranging from 11 to 60% in susceptible cultivars have been reported in Brazil and the United States of America (Paccola-Meirelles *et al*., 2001; Carson, 2005). Therefore, given favourable conditions for disease development in the region, PLS is likely to cause maize yield losses in the magnitude of those observed for other foliar diseases such as GLS, NLB and common rust (Carson, 1999).

In addition, no specific control measures have been reported for PLS disease. In South Africa, curative control using fungicides including those that control GLS, has not been effective (Flett and Lawrance, 2004). Resistant cultivars would therefore be more sustainable and effective as a control measure for increased maize yields, especially in the resource-poor smallholder-farming sector. Preliminary evaluations for PLS resistance in South Africa and Zimbabwe have indicated cultivar variation (Flett and Lawrance, 2004; Mhembere, 2005). This implies that development of inbred lines with adequate levels of resistance to PLS should be possible. Derera *et al*. (2007) also identified some lines that contributed exceptionally high resistance to PLS which could be used as resistant sources.

Maize grey leaf spot (GLS), remains the most important foliar disease in sub-Saharan Africa causing yield losses around 10 to 25% annually (Derera *et al*., 2008; Menkir and Ayodele, 2005). The disease has spread since the 1990s and is now endemic throughout the region (Menkir and Ayodele, 2005). Several studies have been conducted to determine the inheritance of GLS resistance in diverse sources of maize inbred lines, but detailed studies on maternal influences have not been done. For example, Derera (2005) and Menkir and Ayodele (2005), in independent studies, suggested the possible role of maternal effects when they observed large differences between male and female mean squares for GLS resistance. The studies also showed that single cross hybrids would be

[1] George Bigirwa (PLS in Uganda) and Joe DeVries (PLS in Rwanda): AGRA-Nairobi, Eden Square Block 1, 5th Floor, Nairobi, Kenya.

resistant when at least one of the inbred lines carried the resistance to GLS (Derera, 2005; Menkir and Ayodele, 2005). If maternal effects exist, the female line in a single cross involving a susceptible and a resistant line should therefore be resistant, if the levels of resistance are to be enhanced. The role of maternal effects in the inheritance of resistance to GLS needs more investigation, using models that include reciprocal effects.

Production environments in SSA are also highly variable resulting in complicated genotype x environment (G x E) interactions (FAO and CIMMYT, 1997). Performance of hybrids developed should therefore be evaluated in multi-location trials. According to Crossa (1990), multi-location trials are important as they assist in;" *i) accurately estimating and predicting yield based on limited experimental data, ii) determining yield stability and the pattern of response of genotypes or agronomic treatments across environments, and iii) providing reliable guidance for selecting the best genotypes or agronomic treatments for planting in future years and at new sites*". The environments should be both spatial and temporal. Statistical analysis of multi-location trials, in most cases, are able to detect the existence of genotype by environment (G x E) interaction (Fox *et al*., 1997). Genotype x environment interaction is the differential genotypic expression across environments, which has an implication on the breeding strategy that one can adopt, for example, whether to aim for specific or wider adaptation (Fox *et al*., 1997).

Breeders have also often been accused of failing to consider the special preferences of farmers especially those in marginal areas (Toomey, 1999; Banziger and Cooper, 2001), possibly because they are unaware of them. As a result, despite the development of improved, superior cultivars in most of the countries in SSA, the majority of the resource-poor smallholder farmers still rely on unimproved open-pollinated varieties (OPVs) for their plantings (FAO and CIMMYT, 1997; Aquino *et al*., 2001). This has been partly because the OPVs are easy to multiply and therefore cheap and readily available (FAO and CIMMYT, 1997). In addition, most of the breeders of improved cultivars have focused more on raising yields under optimal, agronomically well-managed conditions (Reeves and Cassaday, 2002) and farmers probably perceive little advantage in growing them because they are not designed for their needs (Banziger and Diallo, 2002). Therefore, for effective breeding, farmers' perceived constraints and their preferences for cultivars should be clearly identified through researcher-farmer interaction and collaboration. Farmers can provide vital information on plant types, desired traits and insight into trade-offs they are willing to make among traits in designing cultivar types

(Sperling *et al*., 2001). This enhances the potential for adoption of the varieties in the respective communities where the studies are conducted.

2 Rationale for research focus

The incidence of PLS has increased in maize in the region over the past few years (Carson *et al*., 2005; Vivek *et al*., 2001), and no chemical control measures exist for the disease currently. In addition, in southern and eastern Africa, most maize breeding programmes which are hybrid oriented use elite maize inbred lines from nine heterotic groups (Derera, 2005). These groups include the broader CIMMYT A and B classification (CIMMYT, 2001), the SC, N3 and K64r derivatives and the "P" heterotic group (derivatives from Natal Potchefstroom Pearl) (Gevers and Whythe, 1987; Olver, 1998; Derera, 2005) amongst others. The SC, N3, K64r and P heterotic groups are a significant source of inbred lines used in hybrid production in South Africa and Zimbabwe (Gevers and Whythe, 1987; Cowie, 1998; Olver, 1998). The N3 and SC have been useful in breeding record hybrids such as SR52 that exhibited wide adaptation in east, central and southern Africa. However, the "P" heterotic group, on the other hand, was found to be susceptible to PLS and GLS (Gevers *et al*., 1994; Derera, 2005). Therefore, given the regional significance of the "P" heterotic group, it would be important to improve resistance to PLS as well as GLS in this group for hybrid production and also explore resistance sources in other dominant germplasm backgrounds. In order to develop disease resistant genotypes, an understanding of the genetic variability and inheritance of the resistance is thus important for effective selection to be conducted.

On the other hand, GLS which is now endemic to the region, remains one of the most important and devastating diseases. Although some sources of resistance have been identified from some African adapted germplasm (Gevers *et al*., 1994; Derera *et al*., 2008; Vivek *et al*., 2009), more sources would be useful given the potential of GLS to threaten food security. Useful sources of resistance would be those that can contribute resistance to inbred lines that are susceptible to GLS and are widely used in hybrid production in Africa. In addition, information is still limited on the mode of inheritance of the germplasm that are adapted to African environments (Derera *et al*., 2008). Some studies have suggested the possible role of cytoplasmic effects in GLS resistance based on the differences between male and female mean squares (Derera, 2005; Menkir and Ayodele, 2005). This observation warrants more investigations since a trait that is

completely under maternal effects results in inflated genetic variance, which tends to slow the response to selection (Roach and Wulff, 1987; Hallauer and Miranda, 1988).

Most of the foliar diseases affecting maize in the region occur simultaneously in the field. There is therefore a need to screen germplasm that is already adapted to the region for sources of resistance to PLS disease and the other important foliar diseases, such as GLS, NLB and common rust. This would facilitate the use of the different maize lines in the development of resistant cultivars by the various breeding programmes in the region.

The highly variable environments in SSA contribute to complicated genotype x environment (g x e) interactions. It would therefore be important to determine if g x e interaction exist for PLS and grain yield. A significant g x e would mean that selections from one environment may perform poorly in another (Fox *et al.*, 1997). This would entail breeding for specific adaptation. Genotypes that show little interaction with environments would be desired as they are stable (Tollenaar and Lee, 2002).

Subsequently, to enhance the potential for adoption of varieties by communities, farmers' constraints and their preferences for cultivars need to be identified through researcher-farmer interaction and collaboration and be included in cultivar design.

3 Research objectives

The specific objectives of the study were, therefore:

1. To establish smallholder farmers' key limiting production constraints and desired traits of maize cultivars grown in their specific environments using a rural area in northern KwaZulu-Natal, in South Africa as a case study.
2. To screen germplasm from different heterotic groups used by maize breeding programmes in eastern and southern Africa for resistance to PLS and other important foliar diseases such as GLS, NLB and common rust.
3. To determine the gene action and inheritance of resistance to PLS from six elite tropical maize inbred lines varying in genetic backgrounds.
4. To test for maternal effects in the inheritance of resistance to PLS and GLS in maize hybrids.
5. To estimate combining ability and heterosis of tropical elite maize inbred lines for PLS, grain yield and the other foliar diseases across different environments.

6. To evaluate the performance and grain yield stability of F_1 hybrids of maize derived from crosses between selected elite tropical inbred lines with differential PLS and GLS reactions under different environments in southern and eastern Africa.

4 Research hypotheses

The following hypotheses were tested:

1. Smallholder farmers are aware of the major constraints that affect maize production in their areas and prefer specific traits and stress tolerance levels in their maize cultivars.
2. Adapted regional maize germplasm has wide genetic variability and possesses high levels of resistance to PLS that can be identified and exploited in breeding programmes.
3. There is no epistasis in the African adapted germplasm to render the additive-dominance model inadequate in explaining maize resistance to PLS and GLS.
4. The selected adapted elite tropical maize inbred lines have good combining ability for grain yield and resistance to PLS, GLS, NLB and common rust.
5. Levels of resistance to PLS and grain yield in maize are affected by changes in environment.
6. Maternal effects contribute to the inheritance of resistance to PLS and GLS in maize hybrids.

5 Outline of thesis

The specific objectives mentioned were achieved and are addressed in the various chapters which constitute this thesis. Each chapter is an independent, potential manuscript for journal publication and therefore there may be some overlaps of content and references with other chapters. The chapters are divided as follows:

1. Introduction to thesis
2. Chapter 1: Literature review.
3. Chapter 2: Identification of farmers' key maize production constraints and traits desired in maize cultivars.
4. Chapter 3: Genetic variability of tropical maize germplasm to PLS disease resistance under field conditions.
5. Chapter 4: Combining ability analysis for PLS resistance and agronomic traits in tropical advanced maize inbred lines.

6. Chapter 5: Generation mean analysis of PLS resistance in six tropical advanced maize inbred lines.
7. Chapter 6: Generation mean analysis and combining ability for GLS resistance in elite African maize germplasm.
8. Chapter 7: Diallel analysis of resistance to NLB and common rust diseases in tropical advanced maize inbred lines.
9. Chapter 8: Genotype-environment interaction and grain yield stability of African maize germplasm across different stress environments.
10. Chapter 9: General Overview.

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1 Literature Review

1.1 Introduction

This literature review covers topics relevant to the research focus to provide the theoretical base for the research. It therefore, seeks to give an insight into smallholder maize production and breeding for resistance to phaeosphaeria leaf spot (PLS), other foliar diseases mainly grey leaf spot (GLS), northern corn leaf blight (NLB) and common rust diseases, with emphasis on sub-Saharan Africa (SSA). Information on the importance of PLS, GLS, NLB and common rust diseases in SSA, and the causal organism(s) of PLS is discussed as PLS is a fairly new disease and very little information about it has been published. Combining ability effects including methods used to estimate them and their influence on gene action and implications in plant breeding are covered. The influence of maternal effects on the resistance to PLS and GLS, the implication of genotype x environment and yield stability in breeding is discussed to create an important frame of reference for the research study.

1.2 Constraints limiting maize productivity in sub-Saharan Africa (SSA)

Among the factors limiting maize productivity in SSA are: the production environments, differences in production systems, limited resources, insufficient draught power, poor timing of operations and labour shortages (Mashingaidze and Mataruka, 1992; FAO and CIMMYT, 1997). The majority of the production environments are characterized by inadequate rainfall, low soil fertility, insect pests and diseases (FAO and CIMMYT, 1997).

1.2.1 Production environments and production systems

Most of the maize in SSA is grown under dryland or rainfed conditions (Mataruka, 1985). Supplementary irrigation is applied in some cases only to support the early growth of the crop or when mid-season drought occurs, mostly in large-scale commercial production. This dependence on rainfall by the majority of the farmers has resulted in year-to-year variations in maize yields as most of the maize growing regions are susceptible to drought and there is no irrigation available (Mashingaidze and Mataruka, 1992).

Large differences in maize yields exist in different production systems, for example large-scale commercial versus small scale and subsistence farming. In Zimbabwe, for example, despite the almost 100% adoption rate of hybrid maize in the smallholder-farming sector,

yield on large-scale commercial farms averages over 4.0 t ha^{-1}, compared with around 1.0 t ha^{-1} in the small-scale commercial and subsistence sectors (Mashingaidze and Mataruka, 1992; FAO and CIMMYT, 1997). Most of the differences have been attributed to differences in moisture regimes and soil fertility. In addition, most of the smallholder and subsistence farmers elsewhere in Africa depend heavily on unimproved seed varieties, have limited resources, and inputs for production are also lacking (FAO and CIMMYT, 1997).

1.2.2 Diseases affecting maize in sub-Saharan Africa

The other major constraints are diseases and pests. The diseases which are endemic to most SSA maize production areas include maize streak virus, grey leaf spot (GLS, (*Cercospora zeae-maydis* Tehon & Daniels), rust *(Puccinia sorghi* Schwein. and *P. polysora* Underw.), northern corn leaf blight (NLB, *Exserohilum turcicum* Pass. Leornard & Snuggs), ear rots (*Fusarium* and *Diplodia*), head smuts (*Sphacelotheca reliana*) and Phaeosphaeria leaf spot (PLS, *Phaeosphaeria maydis*) (Bonga and Cole, 1997; Vivek *et al*., 2001). These diseases are often difficult to control since their occurrence year after year is less predictable because of their high dependence on weather. As a result, in favourable seasons with high rainfall, diseases also become more prevalent and damaging. The majority of small-scale farmers cannot afford in most cases, to control the diseases due to limited access to pesticides. Therefore, the development of maize cultivars with enhanced levels of disease resistance and greater abiotic stress tolerance will be more sustainable and effective for increased maize yields, especially in the smallholder-farming sector.

1.3 Occurrence of PLS disease of maize

Phaeosphaeria leaf spot disease is caused by the ascomycete fungus, *Phaeosphaeria maydis* (Henn.) Rane, Payak & Renfro (syn. = *Leptosphaeria zeae-maydis* Saccas; *Metasphaeria maydis* (Henn.) Höhnel), and was first reported from India (Rane *et al*., 1965). The disease occurs throughout the tropics and subtropics, with reports mainly from Central and South America, India, Central, East and Southern Africa, and Hawaii (De Leon, 1984). In Africa, reports have been mainly from Kenya (Njuguna *et al*., 1992), South Africa (Smit and Lawrance, 2004), Zimbabwe (Levy, 1996) and Cameroon (Carson *et. al*., 1991). In South Africa, the disease has been observed most frequently in KwaZulu-Natal, the eastern parts of Mpumalanga and western parts of Gauteng (Flett and Lawrance, 2004). In Zimbabwe, the disease was initially reported around Marondera

area, about 70 km east of Harare (Levy, 1996), but has since spread to most of the high rainfall areas in the highveld and middleveld areas (Mhembere, 2005). However, due to limited literature available on PLS, the actual distribution of the disease in SSA is still unknown.

1.3.1 Incidence of PLS disease on maize

The incidence and severity of PLS has increased over the years causing severe yield losses in susceptible maize cultivars, especially in Brazil (Casela, 1998). In most of the areas where PLS was reported, the disease was initially observed at the end of the season thus not causing any major damages to the maize quality or grain yield (Silva and Moro, 2004). However, the disease seems to have built up slowly over the seasons resulting in significant damage on maize. Grain yield reductions of more than 60% in susceptible cultivars have been reported from Brazil (Cervelatti *et al*., 2002). Studies by Carson (2005) conducted in the USA also reported a reduction in grain yield of 11-13%.

The increase in PLS incidence was reported to be due to practices such as late planting, absence of rotation, and zero tillage practices. In South Africa, Kenya, and Zimbabwe, PLS disease incidence has increased since the early 1990s (Mwangi, 1998; Smit and Lawrence, 2004; Derera *et al*., 2007; Vivek *et al*., 2009). For example, in Kenya PLS incidences of over 85% were recorded in some districts and it is now one of the most important diseases in maize (Mwangi, 1998; Kwena, 2007). The trend towards increasing severity and incidence of PLS in the region (Vivek *et al*., 2001; Carson, 2005; Derera *et al*., 2007) is likely to cause significant damage on maize as has happened in the past with diseases such as GLS (Huff *et al*., 1988; Ward et al., 1999). Significant damage to maize due to PLS disease has already been reported in Brazil demonstrating the potential that it has of becoming a major disease, thereby threatening regional food security. Therefore, it is for these reasons that high priority research should be given to the disease in all the important maize production areas in SSA.

1.3.2 Symptoms of PLS disease on maize

Symptoms of PLS disease start developing on the lower leaves as small, pale green or chlorotic lesions, which become bleached or dried with dark brown margins (De Leon, 1984; Fernandes, 1998; CIMMYT Maize Programme, 2004). The white spots are round, elongate to oblong in shape and are often scattered over the leaf (Fig. 1.1a). Under favourable conditions, the disease can spread rapidly to the young top leaves and

sometimes infect the stems, ear and leaf sheaths (CIMMYT Maize Program, 2004; Flett and Lawrance, 2004). Severe infestations of the disease, especially in late plantings, can result in a considerable reduction in photosynthetic leaf area as the spots coalesce (Fig 1.1b-d) and result in early plant death (Fernandes, 1998).

Figure 1.1 Phaeosphaeria leaf spot disease symptoms on maize (a) white spots scattered all over the leaves, (b) a close-up of the spots that have coalesced, (c) natural infection of experimental hybrids in the field and (d) close-up of a leaf showing severe symptoms of PLS.

1.3.3 Epidemiology of PLS disease of maize

Phaeosphaeria maydis has been found to persist in diseased plant parts in the soil, and the spores germinate under favourable conditions to infect maize leaves (Flett and Lawrance, 2004; Do Amaral *et al*., 2005). Mild temperatures, which are typical of many subtropical regions, favour the survival of this pathogen and this could be associated with an increase in the disease. Practices such as absence of rotation and zero tillage have been reported to promote the increase of inocula over the seasons (Casela, 1998; Cervelatti *et al*., 2002), resulting in increased disease incidence and severity. These practices like zero tillage are common on most commercial farms in Southern Africa,

whereas most resource-poor smallholder farmers, due to limited access to land, do not practice rotation. No other host has been reported for *P. maydis*.

Other conditions favouring PLS disease development include high humidity and relatively low night temperatures (Casela, 1998; Carson, 1999). Studies by Fernandes and Sans (1994) showed a high correlation between disease incidence and plant age, maximum and minimum temperature and relative humidity (RH). They reported that relative night temperatures above 14^{o}C and RH above 70% were adequate for disease development.

1.4 Management of PLS disease of maize

In SSA, currently, no specific chemical control measures exist for PLS disease of maize (Flett and Lawrance, 2004). However, fungicide trials conducted in Brazil demonstrated the effectiveness of mancozeb, a protective fungicide in controlling *P. maydis* when applied before or in the initial phase of disease development (Pinto, 1999). On the other hand, in South Africa, curative control using triazole fungicides, including those that control GLS, has not been effective (Flett and Lawrance, 2004). Therefore, the use of resistant cultivars should form the most economic and efficient method of disease management especially for the resource-poor smallholder farmers since maize has great genetic diversity for resistance to pathogens (Duvick, 1984; Silva, 2001).

1.5 Breeding for resistance to PLS disease of maize

Research done in Brazil, India, South Africa and Zimbabwe has shown significant differences in the resistance of inbreds, experimental hybrids and open-pollinated varieties (OPVs) to PLS disease (Das *et al*., 1989; Casela, 1998; Carson, 1999; Paterniani *et al*., 2000; Pegoraro *et al*., 2002; Silva and Moro, 2004; Smit and Lawrence, 2004; Derera *et al*., 2007). This demonstrates that genetic variability to PLS resistance is available. However, the inbred line B73 and its derivatives (commonly used as female (seed) parents in hybrid seed production in the United States) were found to be susceptible to PLS compared to those belonging to the other heterotic groups such as the “Lancaster” (Carson, 1999). Other resistant sources from Brazil included DAS95, DAS41 and DAS86 derived from various populations such as the Tropical flint synthetic, Suwan DMR and Amarillo dentado/ Carribean flint (Silva and Moro, 2004). Though, these resistant sources are available, maize germplasm that performs well in temperate regions such as the United States generally cannot be introduced into non-temperate regions without undergoing extensive testing and selection for local adaptation (Morris, 2002).

Therefore, most of the improved varieties grown in the United States are of little direct use in developing countries. In addition the resistant germplasm from Brazil is predominantly orange to yellow, whereas the majority of farmers in SSA prefer white maize (FAO and CIMMYT, 1997). This implies that the resistant germplasm from Brazil would also have to undergo extensive local breeding and testing for adaptation and conversion before it can be used in hybrid production.

In SSA, preliminary studies for PLS resistance have been conducted in South Africa, and Zimbabwe and these studies showed significant cultivar variation (Flett and Lawrence, 2004; Smit and Lawrence, 2004; Derera *et al*., 2007). The inbred lines that contributed high resistance to PLS in studies by Derera *et al*. (2007) included; B23, B17, B12 and CML444. However lines belonging to the P heterotic group were observed to be more susceptible to PLS, whereas those from the K group were mostly resistant (Derera *et al*., 2007). Olver (1998) indicated that most of the early hybrids in the region were constituted from lines belonging to the P, K and B73-type germplasm. Therefore as the disease continues to increase in incidence and severity in the region, more sources of resistance are needed, especially in germplasm from other genetic backgrounds that can be used as parents in hybrid production.

1.6 Genetics of resistance to PLS disease of maize

The development of disease resistant genotypes depends upon an understanding of the genetic variability and inheritance of the resistance for effective selections to be conducted. Different types of gene action that controls the inheritance of resistance to PLS have been reported by various authors and these are discussed below.

Additive gene action was shown to be more important for PLS disease inheritance than non-additive gene action (Carson, 2001; Paterniani *et al*., 2000; Pegoraro *et al*., 2002; Derera *et al*., 2007; Vivek *et al*., 2009). Studies by Paterniani *et al*. (2000) and Derera *et al*. (2007) indicated that general combining ability (GCA) effects contributed more than 90% to the variation in PLS resistance in southern African and Brazilian maize. Additional studies using a diallel mating design for 12 inbred lines adapted to African conditions, reported that GCA contributed 65% and specific combining ability (SCA), 35% of the variation in PLS resistance (Vivek *et al*., 2009). These studies led to the conclusion that PLS was predominantly controlled by genes with additive effects. On the other hand,

Silva and Moro (2004) observed highly significant GCA effects in a diallel study involving nine lines adapted to Brazilian conditions, the SCA effects were non-significant implying non-additive genes were not important in these inbred lines. In contrast, Das *et al*. (1989) using a diallel cross of eight open pollinated varieties of maize found significantly higher levels of dominance variance than additive effects on the genetic control of PLS disease resistance, an indication that non-additive gene action was more important than additive gene effects.

PLS resistance was also shown to be inherited quantitatively and conditioned by genes that exhibited incomplete dominance (Carson *et al*., 1996; Carson, 2001). Estimates of the number of genes involved in the inheritance of resistance ranged from three to four loci (Carson, 2001). In contrast, Pegoraro *et al*. (2002) observed two major independent genes that were involved in the inheritance of resistance to PLS disease.

However, in most of these studies there was no separation of the non-additive effects into dominance and epistasis components. Carson (2001), used generation mean analysis (GMA) for B73 x Mo17 cross to partition the non-additive effects. The study reported highly significant additive gene action, with dominance genetic effects accounting for only less than 10% of the variation in PLS resistance among the generation means (Carson, 2001). This implied that the additive-dominance model was adequate in explaining PLS disease resistance in the B73 x Mo17 cross, since no epistasis was observed. However, GMA relies on a fixed model, where the inference applies only to the materials involved in the study, thus it is possible that epistasis could be present for PLS resistance in other maize populations. Presence of epistasis would render the additive-dominance model inadequate and would therefore influence the breeding strategy that can be used. Therefore, germplasm that dominate hybrid parentage in germplasm adapted to African environments needs to be investigated for the gene action controlling inheritance to PLS disease.

The importance of additive gene action for PLS resistance was also confirmed by the relatively high estimates (60 to 85%) of broad- and narrow sense heritability (Carson *et al*., 1996, 2005; Mhembere, 2005; Derera *et al*., 2007). Therefore these high heritability estimates and highly significant additive gene action reported for PLS imply that selection for resistance to PLS would be very effective. However, the significant non-additive effects, especially dominance found in some of the populations should not be overlooked as this may slow progress. Presence of epistasis and dominance in the African maize

germplasm needs to be investigated since this directly influences the breeding strategy for PLS resistance.

1.7 Importance of maize grey leaf spot disease in SSA

Maize grey leaf spot (GLS) disease is currently the most important foliar disease in SSA resulting in yield losses around 10 to 25% annually, although losses as high as 90% due to severe deterioration of the leaves and stalk lodging have also been recorded (Latterell and Rossi, 1983). The disease has spread since the 1990s and is now endemic throughout the region (Menkir and Ayodele, 2005). Grey leaf spot development depends mainly on the availability of inoculum, relative humidity (RH) of 100% and temperature between 22 and 30^{o}C (Beckman and Payne, 1982; Thorson and Martinson, 1993). The disease usually develops from tasseling stage and thus perceived as a "late-season" disease but infection may occur prior to tasseling stage (Ward *et al*., 1999).

Disease symptoms on mature leaves occur as tan to brown, 5 to 60 mm long, narrow lesions with parallel sides and squared-off ends (Ward *et al*., 1993). As the number of lesions increase, the spots coalesce resulting in larger blighted areas and dense sporulation producing a greyish cast. Heavy infestations can result in ear husk and stalk lesions occurring leading to lodging (Ward *et al*., 1993). Early blighting of leaves above the ear causes severe yield loss, while blighting after grain-fill causes little loss.

1.8 Breeding for resistance to GLS in maize

Sources of resistance to GLS have been identified and reported by many researchers (Thompson *et al*., 1987; Donahue *et al*., 1991; Gevers *et al*., 1994; Coates and White, 1998; Menkir and Ayodele, 2005; Pratt and Gordon, 2006; Derera *et al*., 2008; Vivek *et al*., 2009). Most of these sources which include Mo18W, NC250, NC250A, NC258, NC290, Pa875, Va59 and Oh43 (Freppon *et al*., 1994; Pratt and Gordon, 2006) are temperate materials and cannot be used directly in African tropical environments without undergoing extensive breeding and testing for local adaptation. Other sources of resistance have been identified from some African adapted germplasm including the white modified *opaque*-2 maize (KO54W and SO507W) belonging to the F and M heterotic groups, respectively in South Africa (Gevers *et al*., 2004). However, these modified *opaque*-2 maize are not adapted to tropical conditions in SSA. Gordon *et al*. (2004) also characterized a South African inbred line VO613Y as resistant to GLS. In

addition, Derera *et al*. (2008) identified some resistant sources in heterotic groups A, N3, B, K and SC which are adapted to tropical conditions. These were mainly A13, A15, B18 and B19 inbred lines. However, given the significant damage caused by GLS in susceptible maize cultivars, more sources of resistance would be useful, especially those that can contribute resistance to susceptible inbred lines that are widely used in hybrid production in Africa. It appears GLS will remain an important disease in the region as a result of minimum tillage practiced by most commercial farmers. Most of these commercial farmers rely on chemical control of GLS disease, whereas resource-poor smallholder farmers cannot afford these chemicals. Therefore resistant cultivars are necessary for deployment to SH farming areas which are prone to GLS epidemics.

1.9 Genetics of resistance to GLS

Research done on the mode of gene action for resistance to GLS using diallel crosses and generation mean analysis has indicated resistance to be controlled mainly by genes with additive effects (Thompson *et al*., 1987; Huff *et al*., 1988; Elwinger *et al*., 1990; Ulrich *et al*., 1990; Gevers and Lake, 1994; Coates and White, 1998; Menkir and Ayodele, 2005; Derera *et al*., 2008; Vivek *et al*., 2009). Some of these reports suggested that non-additive effects, particularly dominant gene action also played a role in the resistance (Elwinger *et al*., 1990; Coates and White, 1998; Derera *et al*., 2008; Vivek *et al*., 2009). Other studies by Thompson *et al*. (1987) and Ulrich *et al*. (1990) even reported 100% GCA contribution to the hybrid variation for GLS resistance, implying that in the germplasm tested only additive gene action was important. Hohls *et al*. (1995) reported that resistance was conditioned by additive and complete dominance with minor epistasis in maize lines from three divergent backgrounds in South Africa.

Broad-sense heritability estimates reported for GLS resistance ranged from 64 to 92% (Clements *et al*., 2000; Vivek *et al*., 2001; Cromley *et al*., 2002; Derera *et al*., 2008). In work done by Gordon *et al*. (2006), heritability based on broad sense values ranged from 46 to 81% depending on the disease severity and incubation period. Two or more effective factors (minimum number of genes) were reported to condition the resistance in populations tested from American germplasm (Coates and White, 1998; Pratt and Gordon, 2006). Negative mid-parent heterosis exceeding 10% was reported for GLS resistance by Menkir and Ayodele (2005) and Derera *et al*. (2008) in some crosses which involving susceptible and resistant parents. Cromley *et al*. (2002) also reported similar results when crosses were made between resistant and susceptible parents. This

confirmed the presence of genes with non-additive effects in the inbred lines used. The results further confirmed that adequate GLS resistance could be obtained in single cross hybrids when one parent was resistant (Derera *et al*., 2008). It would be important therefore to screen germplasm for more resistant sources in African germplasm that can be used in hybrid production.

1.10 Importance of northern corn leaf blight (NLB) disease in SSA

Northern corn leaf blight (NLB) is amongst the most common and widespread maize leaf disease worldwide (Vivek *et al*., 2009). It is caused by *Exserohilum turcicum* (Pass) Leornard and Suggs (teleomorph = *Septosphaeria turcica* Leornard & Suggs. Syn. = *Helminthosporium turcicum*) (Smith and White, 1988). Symptoms range from small elliptical shaped lesions to complete destruction of the leaves (Welz and Geiger, 2000). The disease is prevalent in areas with prolonged dew periods and moderate temperature, and these conditions are common in most production environments in eastern and southern Africa (CIMMYT Maize Program, 2004). The pathogen survives in crop residues and initiates disease epidemics with conidia on these residues (Robert and Findley, 1952).

Northern corn leaf blight can cause severe defoliation during grain-filling period resulting in grain yield losses of more than 50% in susceptible cultivars (Perkins and Pedersen, 1987; Raymundo and Hooker, 1981). The disease is mainly controlled by resistant cultivars through both qualitative (race-specific) and quantitative (non-race specific) resistance.

1.11 Breeding for disease resistance to NLB in maize

Sources of both qualitative and quantitative resistance to NLB are available (Welz and Geiger, 2000). However, qualitative resistance is unstable and breaks down easily as new virulent strains come in. In the tropical environments most breeders have focused more on quantitative resistance, which is more durable (Sharma and Payak, 1990; Paliwal, 2000). High levels of quantitative resistance have been demonstrated in inbred lines such as H99 (Lipps *et al*., 1997; Hakiza *et al*., 2004). Brewbaker *et al*. (1989) reported some highly resistant lines adapted to tropical and temperate conditions and these included CM118 (India), Fla2AT116 (Florida), ICA 127 (Columbia), H55 (Indiana), Hi39 (Hawaii), and F (Kenya; a parent of hybrid H632). The CIMMYT line CML202 was shown to provide quantitative resistance and is adapted to most African environments

(Schechert *et al*., 1997, 1999). Other CIMMYT lines such as CML443, CML444 and CML445 were reported to be resistant and these lines are adapted to mid-altidude conditions (CIMMYT, 2004).

However, despite the availability of these resistant sources, lately there has been a resurgence of the NLB disease in the region. Vivek *et al*. (2009) also reported that incidence and severity of NLB had increased especially in Southern Africa in the past three years. It could be possible that most of the resistance that was available in some of the regional hybrids was probably qualitative and this is not stable. Another speculation is that there could be more temperate susceptible germplasm being introduced into these tropical environments, thus giving rise to increased NLB disease severity. In southern Africa, commercial farmers also rely on chemical sprays for NLB and this could lead to development of strains that are resistant to the chemicals and therefore cause significant damage on the maize. It is important therefore to identify more sources of quantitative resistance to NLB, which is durable.

1.12 Genetics of resistance to NLB

Quantitative resistance for NLB has been shown to be inherited polygenically (Pataky *et al*., 1986). Additive gene action was found to be of major importance in almost all the studies on the quantitative inheritance of NLB (Sigulas *et al*., 1988; Carson, 1995; Schechert *et al*., 1997; Vivek *et al*., 2009). However, Schechert *et al*. (1997) observed a changing gene action depending on the developmental stage of the maize, with the SCA effects becoming more important with progressing plant growth. Studies on NLB inheritance by Vivek *et al*. (2009) showed a contribution of 61% and 39% for the GCA and SCA effects, respectively. Quantitative resistance to NLB was highly heritable in general, although low (26%) to high (89%) broad-sense heritability estimates were obtained in some trials (Hughes and Hooker; 1971, Carson, 1995; Welz *et al*., 1998, Schechert *et al*., 1999). It is therefore important to study the gene action in the new materials being screened for NLB resistance.

1.13 Importance of rust disease in SSA

The two main rust pathogens affecting maize in SSA are *Puccinia sorghi* (common rust) and *P. polysora* (lowland or tropical rust) (CABI, 1970). Common rust thrives under cool and humid conditions, whilst the tropical rust is favoured by warm, humid conditions (Vivek *et al*., 2009; CIMMYT Maize Program, 2004). Therefore, common rust tends to be

widespread in subtropical, mid-altitude and temperate environments, whereas, the tropical or lowland rust thrives in tropical and subtropical regions (CABI, 1970, Renfro and Ullstrup, 1976; Brewbaker, 1979). Common rust has been reported to be common and widespread on maize in South Africa (Craven *et al*., 2007) and Vivek *et al*. (2009) also indicated it was amongst the diseases that are now endemic to SSA.

Symptoms caused by the two rusts are almost similar, but can be distinguished based on the size, shape and colour of the pustules (Scott et al., 1984). *Puccinia polysora* occurs predominantly on the upper surface of the leaf, whereas, *P. sorghi* occurs abundantly on both leaf surfaces (CIMMYT Maize Program, 2004). The symptoms are small, circular to elongate, powdery uredial pustules varying from orangish to dark red/brownish in colour, which later turn black on the development of the telial stage (McGee, 1990). The rust diseases can cause yield losses in excess of 45%, especially where maize is cultivated continuously (Brewbaker, 1974; Kim and Brewbaker, 1976; Raid *et al*., 1988). The diseases are effectively controlled through use of resistant cultivars (Bergquist and Pryor, 1984).

1.14 Breeding for disease resistance to rust

Both qualitative and quantitative resistance are available (Robert, 1962; Hooker and Saxena, 1971; Bergquist and Pryor, 1984). More than 25 dominant rust-resistant genes have been reported in maize (Hu and Hulbert, 1996). Quantintative or general resistance is the most preferred type of resistance in most tropical environments as it is more durable. Local farmers in East and West Africa were reported to have incorporated quantitative resistance in their cultivars through mass selection (Pataky, 1999; Paliwal, 2000; Pratt and Gordon, 2006). Some sources of resistance to rust that have been identified for quantitative resistance include Oh545, CM111, CM105 (Kim and Brewbaker, 1977), and highland-adapted lines such as CML239 and CML246 (CIMMYT, 2004). More sources of resistance are essential, especially for quantitative resistance. For that reason it is vital to identify and incorporate quantitative resistance in most of the germplasm adapted to African environments as it is more durable.

1.15 Genetics of resistance to rust

Highly significant GCA effects were reported for rust indicating the importance of additive gene action (Kim and Brewbaker, 1977; Paterniani *et al*., 2000; Vivek *et al*., 2009).

Although dominance was also significant in all these studies, its contribution was small. Paterniani *et al*. (2000) reported that GCA effects for leaf disease severity scores accounted for 94% of the total variation, whilst SCA effects, though significant accounted for only 6% of the variation. Vivek *et al*. (2009) reported 70% and 30% GCA and SCA contributions, respectively, to total variation for rust resistance. Scott *et al*. (1984) reported that the type of gene action for *Puccinia polysora* (lowland or tropical rust) included complete, partial or no dominance in five different resistant selections crossed to a susceptible tester. Kim and Brewbaker (1977) reported estimates of broad and narrow sense heritability ranging from moderate to high (47 to 97%).

1.16 Association of PLS Resistance with other Maize Diseases

Resistance genes to different pests and pathogens have been reported to be clustered in the maize genome (McMullen and Simcox, 1995, Wisser *et al*., 2006). A synthesis of different publications on the mapping of maize disease resistance loci, reported the locations of 437 quantitative trait loci (QTL) for disease (dQTL), 17 resistance genes (R-genes) and 25 R-gene analogues (Wisser *et al*., 2006). Based on this review, the presence of clusters of dQTL for multiple diseases was identified and from the distinct dQTL distributions for the different diseases, it was evident that certain breeding schemes would more suitable for certain diseases (Wisser *et al*., 2006). It may therefore be possible to breed for multiple disease resistance especially if the breeding schemes are the same. For example, the numerous QTL mapped for NLB were scattered over the genome, implying that a large number of loci contributed to NLB resistance (Wisser *et al*., 2008) and several studies using recurrent selection reported increased NLB resistance (Ceballos et al., 1991; Campana and Pataky, 2005; Carson, 2006). This is an indication of oligogenic mode of inheritance (Ceballos *et al*., 1991).

For PLS disease, five QTLs on different chromosomes were found to control resistance in Mo17 (Carson *et al*., 2005). When these QTLs were compared with previously mapped maize disease and pest resistance, several associations were observed. Examples of these associations are indicated in Table 1.1. However, despite these associations between reported QTL for resistance to PLS and other pests and pathogens, there is no evidence that these resistances are actually correlated.

Table 1.1. Associations between QTLs for resistance to PLS and other maize pests and pathogens

Chromosome	PLS Chromosome bin	Associated pathogen/pest and loci	Reference
One	1.06	Hm1 locus for resistance to *Cochliobolus carbonum* R.R. Nelson, race 1	Johal and Briggs, 1992
Four		Same as QTL for resistance to *Cercospora zeae-maydis* Tehon & Daniels	Bubeck *et al*., 1993 Saghai *et al*., 1996
Seven	7.03	Same region as QTL for resistance to i) *Exserohilum turcicum* (Pass.) Leonard and Snuggs	Dingerdissen *et al*., 1996
		ii) second brood European corn borer	Beavis *et al*., 1994
		iii) sugarcane borer	Bohn *et al*., 1996

1.17 Estimating gene action

Gene action can be obtained by evaluating progenies developed through various mating designs. According to Hallauer and Miranda (1988), all mating designs include progenies that involve relationships among relatives having known genetic components of variance. Specific mating designs can be used to estimate the effects of general combining ability (GCA) and specific combining ability (SCA) in addition to the different genetic variances (Stuber, 1980; Christie and Shattuck, 1992; Singh, 1993). Gene action is then deduced through estimates of GCA and SCA variances and effects (Singh, 1993). These mating designs are also frequently used in inbred and hybrid development programmes (Bernardo, 2002).

The most common mating designs that have been used include diallel (Griffing, 1956; Geraldi and Miranda, 1988) and biparental crosses commonly referred to as North Carolina designs I, II, and III (Comstock and Robinson, 1948; Singh, 1993). In this study, the diallel mating design was used as it allows crosses among all possible combinations from a group of parents including the parents themselves (Jinks and Hayman, 1953; Bernardo, 2002). In addition estimates of GCA, SCA and other effects (Hayman, 1954; Griffing, 1956) can be obtained.

The GCA measures the average performance of a line in all its crosses and this is expressed as a deviation from the overall mean of all the crosses (Hallauer and Miranda, 1988; Christie and Shattuck, 1992; Falconer and Mackay, 1996). However, a line can be crossed to several others, and the expected value of the cross that is predicted from the GCAs of its two parental lines deviates from the observed value (Christie and Shattuck, 1992; Hallauer, 1992). This deviation is called the SCA of the cross (Sprague and Tatum, 1942; Christie and Shattuck, 1992). In general, SCA shows those situations in which the performance of a hybrid is relatively better or worse than would be expected on the average performance of the parents involved (Sprague and Tatum, 1942; Hallauer, 1992).

The GCA is recognized as a measure of additive gene action, whilst the SCA is an estimate of non-additive gene action (Sprague and Tatum, 1942; Bhuller *et al*., 1979). A relatively large GCA/SCA variance ratio suggests the importance of additive gene effects and a low ratio implies the presence of dominant and/or epistatic gene effects (Sprague and Tatum, 1942; Griffing 1956, Bhuller *et al*., 1979).

1.18 Generation mean analysis

Although there are several methods for estimating gene action that are covered in detail by several authors (Stuber, 1980; Christie and Shattuck, 1992; Singh, 1993; Hallauer and Miranda, 1988), in this current study generation mean analysis (GMA) was also used. Generation mean analysis gives information on the relative importance of additive, dominance and epistatic effects in a cross between two inbred lines and is especially powerful in partitioning the epistasis effects (Bernardo, 2002). The method involves measuring the mean of different generations derived from two inbreds and interpreting the means in terms of different genetic effects (Hayman, 1958; Gamble, 1962; Bernardo, 2002). Usually only additive and dominance effects are assumed present and only parent 1 (P_1), parent 2 (P_2) and the F_1 generations are used in this case (Mather and Jinks, 1982; Chahal and Gossal, 2002). However, any departure in the observed and expected values of these generations would indicate the presence of non-allelic interactions, which would then require six generations (P_1, P_2, F_1, F_2 and backcross (BC) generations BCP_1, BCP_2) to be used for the estimations.

Generation mean analysis has the advantage that the populations used provide generations that can be used in a breeding programme (Coates and White, 1998). All the

genetic effects can be estimated simultaneously and because they are estimated from the generation means rather than variances, their sampling errors are inherently smaller than the variances for additive, dominance and epistatic interactions (Bernardo, 2002).

Generation mean analysis is most useful when the parents are divergent, that is, when most, if not all of the favourable alleles are in one parent and unfavourable alleles are in the other parent (Bernardo, 2002). As a result it has been commonly used to study disease resistance where one parent is highly resistant and the other parent highly susceptible. In maize, GMA has been used in several disease resistance studies, some of which include; *Kabatiella zeae* (Reifschneider and Arny, 1983), GLS (Coates and White, 1998; Cromley *et al*., 2002), anthracnose leaf blight (Carson and Hooker, 1981a), stalk rot caused by *Colletotrichum graminicola* (Carson and Hooker, 1981b), NLB (Hughes and Hooker, 1971), brown spot caused by *Physoderma maydis* (Moll *et al*., 1963), common rust (Kim and Brewbaker, 1977), PLS (Carson, 2001) and aspergillus ear rot and aflatoxin (Campbell and White, 1995).

The GMA conducted for PLS was applied only to the cross between B73 and Mo17 and its generations (Carson, 2001). From this analysis, Carson (2001) concluded that the simple additive-dominance model was adequate in explaining the resistance to PLS in this cross. There was no epistasis observed, or transgressive segregation in the F_2 generation (Carson, 2001). However, the results are applicable only to the cross B73 x Mo17. This current study has, however, focused on investigating the gene action for PLS in more populations and their reciprocal crosses as well as to test if maternal effects are also important.

Most of the gene action studies conducted for GLS were based on the GCA and SCA effects estimated from various mating designs. These studies did not separate the additive from the non-additive gene action. However, Coates and White (1998) and Cromley *et al*. (2002) applied GMA to some populations involving crosses using American temperate germplasm. In both instances, the additive-dominance model was reported to be sufficient in explaining the resistance to GLS in the populations tested (Coates and White, 1998; Cromley *et al*., 2002). However, Coates and White (1998) indicated there were some populations which had significant epistasis when the late disease rating scores were used. It appears, therefore, that epistasis could be important in GLS disease resistance, but this still needs verification. Therefore, this current study investigated these non-allelic interactions in populations involving a number of crosses

between resistant and susceptible lines adapted to African tropical environments and the reciprocal crosses were also included in the GMA model.

The main disadvantage of GMA is that it focuses on one trait at a time and because one selects the parents, the inferences are restricted only to the inbred lines involved in the cross. In addition, if the inbred lines have comparable means for the trait, GMA becomes of limited use. Variation among individual plants in each generation has also been used to estimate additive and dominance variances, which in turn have been used to obtain heritability estimates (Mather and Jinks, 1982).

1.19 Role of maternal effects in resistance to diseases

There have been suggestions on the possible role of maternal effects in influencing resistance to PLS and GLS. For example, independent studies by Menkir and Ayodele (2005) and Derera *et al*. (2008) reported large differences between male and female mean squares for GLS resistance. Derera *et al*. (2007) also reported the predominance of the female GCA over the male GCA for PLS resistance in the southern African germplasm. All this suggested the influence of cytoplasmic inheritance and this warrants further investigations. However, these conclusions were only based on the male and female GCAs, and no reciprocal crosses were made. For the other diseases, such as NLB, no maternal or cytoplasmic effects were observed in studies by Sigulas *et al*. (1988) or Schechert *et al*. (1997).

Differences in reciprocal crosses have been used as the most direct evidence for unequal contribution by maternal and paternal parents to phenotype of offspring (Roach and Wulff, 1987). In this case, pairs of individuals serve as both maternal and paternal parent. Reciprocal pairs have similar nuclear genetic contribution and any difference in performance of reciprocal pairs will be due to maternal (or perhaps paternal) effect (Cockerham and Weir, 1977; Roach and Wulff, 1987).

Investigation of maternal effects is important because for a trait that is completely under maternal effects (that is, cytoplasmic or genetic), the amount of genetic variance would be inflated and this tends to slow the response to selection (Roach and Wulff, 1987; Hallauer and Miranda, 1988). In addition, presence of maternal effects influences the choice of female line in single cross hybrids, that is, the female should be the resistant line, if the levels of resistance are to be enhanced.

1.20 Genotype x environment interaction and stability analysis

The environment tends to have a great effect on quantitative than qualitative traits (Mather and Jinks, 1982; Dabholkar, 1992; Singh, 1993; Falconer and Mackay, 1996; Bernardo, 2002). Genotypes also respond differently to changes in environmental conditions and selections from one environment often perform poorly in another. This gives rise to genotype x environment interactions (GEI) which can affect the efficiency of a selection programme as they influence the estimation of variance components (Sprague, 1966). Genotype x environment interaction (GEI) is defined as the differential expression of genotypes across environments (Fox *et al*., 1997). There are different types of GEI which include genotype x location interaction (GLI), genotype x year interaction (GYI) and genotype x location x year interaction (GLYI) (Crossa, 1990). These interactions have different effects with some causing changes in the ranking of the genotypes in different environments, while some result in the genotypes behaving differently but without changes in the rank order in the different environments (Crossa and Cornelius, 1977; Bernardo, 2002). A change in rank order is defined as cross-over interaction and is a major problem in breeding (Cooper and Delacy, 1994; Crossa *et al*., 1995), because it can slow down selection progress as different cultivars are selected in different environments.

Breeders mostly desire genotypes that show little interaction with the environment as they are stable (Tollenaar and Lee, 2002). Stability can be static or dynamic (Becker and Leon, 1988; Bernardo, 2002). Static stability results in the performance of the genotype not changing even when the environmental conditions change. On the other hand, dynamic stability is when the performance of the genotype is affected by the environment but its performance is consistent across environments (Bernardo, 2002).

1.20.1 GEI observed for the foliar diseases

Severity of most diseases tends to vary with locations or environments resulting in significant GEI (Levy and Pataky, 1992; Carson, *et al*. 1997). This is because many pathogens are sensitive to environmental changes. Vivek *et al*. (2009) reported significant GEI for PLS, GLS, NLB and common rust and they attributed this mainly to differences in disease pressure influenced by the prevailing weather conditions in the different environments. Despite these differences, there was a positive correlation between GLS scores with the environments, implying that evaluation of this disease in any of the

environments would be sufficient for selection of resistant germplasm (Vivek *et al*., 2009). In other words, most of the GEI observed for diseases are not of the cross-over type. Carson *et al*. (2002) also observed GEI for GLS which was a result of changes in the magnitude of differences between hybrids when inoculated with the GLS isolates and not changes in hybrid ranking. Derera *et al*. (2008) and Lipps *et al*. (1998) in independent studies also reported GEI for GLS where the hybrid ranking remained the same and only disease severity at the different locations and years contributed to the interactions.

For PLS, a significant genotype x environment (g x e) x time of disease assessment interaction, both under dryland and irrigated conditions was shown by Smit and Lawrance (2004) in their cultivar evaluations over all seasons. However, most of this interaction appeared to be caused by variation in PLS severity and prevailing weather conditions during the four seasons of the study (Smit and Lawrence, 2004). On the other hand, studies conducted in the USA over 2 years, based on 158 recombinant inbred lines derived from the B73 x Mo17 cross, showed that GEI was of minor importance in resistance to PLS disease (Carson, 2001; Carson *et al*., 2005). Similar results were obtained in preliminary studies of PLS resistance conducted at two locations, Rattray Arnold, Zimbabwe and Cedara, South Africa (Mhembere, 2005). According to Mhembere (2005) there was a strong positive correlation for PLS scores with the two locations. An insignificant GEI for PLS disease resistance implies stability of the trait across multiple environments. Therefore, selections from one environment would be expected to perform consistently well in other environment (Fox *et al*., 1977). Given the highly variable production environments in Africa, it is therefore, important to investigate the presence of GEI for diseases in the regional germplasm. This will enable breeding programmes to decide on whether to screen at one site or do multi-location disease screening, which may have financial implications.

1.20.2 Methods used for exploring GEI

There are different ways of dealing with GEI (Bernardo, 2002). Multi-environmental trials (METs) can be used to identify varieties which are superior based on their mean performance across all environments and these can be recommended to farmers (Bernardo, 2002). However, this implies that cultivars selected as superior may not necessarily be the best ones available for a specific environment. METs can also assist in the identification of production environments that best suit certain genotypes (Yan *et al*., 2001). One can also breed for specific adaptation, which involves identifying cultivars best suited for specific environments to maximize productivity (Fox *et al*., 1997; Bernardo,

2002). Another way of dealing with GEI is to select homogeneous subgroups of environments (that is, environments with similar soil types, temperature, rainfall, day lengths, biotic and abiotic stresses) and make recommendations for the different subgroups (Bernardo, 2002).

Various methods have been used to explore GEI and identify superior genotypes with wide or specific adaptation for different environments. These include non-parametric methods for measuring stability (Hill, 1975; Lin *et al*., 1986; Becker and Leon, 1988; Crossa, 1990). Other methods include linear regression (Finlay and Wilkinson, 1963; Eberhart and Russell, 1966; Perkins and Jinks, 1968; Freeman and Perkins, 1971) and principal component analysis (Hill and Goodchild, 1981). However, currently most breeders are using the additive main effects and multiplicative interaction (AMMI) analysis (Gauch, 1992; Gauch and Zobel, 1997) and the genotype and genotype by environment (GGE) biplot analysis (Yan and Kang, 2003; Yan *et al*., 2007).

In the AMMI model, the main effects are retained as additive effects, while the GEI is treated as a multiplicative effect (Gauch, 1988). The AMMI procedure utilizes an analysis of variance (ANOVA) for the effects due to genotypes and environments, and principal component analysis for the GEI (Bernardo, 2002). The objective of AMMI analysis is to obtain an improved estimate of the performance of a genotype in a particular environment (Gauch, 1992). Biplots have been used with AMMI analysis for visually interpreting the performance of genotypes in different environments (Bradu and Gabriel, 1978; Kempton, 1984).

The GGE biplot analysis is based on singular value decomposition (SVD) of environment-centred or within-environment genotype-by-environment data (GED) (Yan *et al*., 2000; Yan *et al*., 2007). The biplots display both genotype (G) and genotype x environment interactions (GEI), which are the two sources of variation that are relevant to cultivar evaluation (Kang, 1993; Yan and Kang, 2003). The GGE biplot is a visual tool that graphically displays GEI from a two way table (Yan *et. al*., 2000). The GGE biplot can be effectively used for mega-environment analysis to show genotypes for specific environments (Yan *et al*., 2007). In addition, it can be used for genotype evaluation (where the mean performance and stability of genotypes can be deduced) and for environmental evaluation (inter-relationships among environments). In this study both the AMMI and GGE biplot analyses were used.

1.21 Farmers' preferences and participatory research

Breeders have often been accused of failing to consider the special preferences of farmers especially those in marginal areas (Toomey, 1999; Banziger and Cooper, 2001), possibly because they are unaware of them. As a result, despite the development of improved, superior cultivars in most of the countries in SSA, the majority of the resource-poor smallholder farmers still rely on unimproved open-pollinated varieties (OPVs) for their plantings (FAO and CIMMYT, 1997; Aquino *et al*., 2001). This has been partly because the OPVs are easy to multiply and therefore cheap and readily available (FAO and CIMMYT, 1997). In addition, most of the breeders of improved cultivars have focused more on raising yields under optimal, agronomically well-managed conditions (Reeves and Cassaday, 2002) and farmers either perceive little advantage in growing them because they are not designed for their needs (Banziger and Diallo, 2002). Therefore, for effective breeding, farmers' perceived constraints and their preferences for cultivars should be clearly identified through researcher-farmer interaction and collaboration.

Farmer participatory research has been defined as the "*collaboration of farmers and scientists in agricultural research and development*" (Bentley, 1994). Participatory methods are now preferred as they recognize the value of farmers' local knowledge, their interests and ability to experiment and innovate, and their active exchange of information and technologies as well as the fact that the farmers are not a homogeneous group — they have different preferences and priorities (Bellon, 2001).

Small-scale farmers' have been involved in plant breeding at various levels of the breeding process. Farmers in Southern Africa, for example participated in evaluation of pre-selected cultivars in CIMMYT's (International Maize and Wheat Improvement Centre) mother-baby trials (Banziger and de Meyer; 2002). In Ethiopia, studies by Abebe *et al.* (2005) also revealed that, generally farmers have their own way of selecting a variety for their localities, although in some cases the farmers' preferences coincide with the breeders' selection. It is, therefore, important to determine from farmers their preferred traits in crop varieties or include the farmers in a variety selection process. This enhances the potential for adoption of the varieties in the respective communities where the studies are conducted.

1.22 Summary

From the review of literature, yields are still low in the smallholder farming sector in SSA. In the majority of SSA countries, landraces and unimproved varieties still form the most common type of seed used by the farmers, despite the advantages of using improved varieties. Farmers either do not see the advantage in growing these improved varieties because they are not designed for their needs. Therefore, breeders should consider farmers' preferences in their breeding programmes or involve the farmers in the selection process at some stage to increase the chances of adoption of improved varieties.

Although PLS is not mentioned in the available literature among the dominant constraints limiting maize yields in sub-Saharan Africa (SSA), the disease has great potential to threaten regional food security. Substantial grain yield losses correlated with PLS severity were reported in Brazil, indicating that severe infestations from PLS can result in considerable yield reductions and even early plant death. The disease therefore needs the attention of both breeders and pathologists in the maize industry. In addition, there are other foliar diseases such as GLS, NLB and common rust which are important in SSA and infect maize together with PLS. It is thus important, that common sources of resistance to these major diseases be found. Presently significant differences in resistance of inbreds, open-pollinated varieties (OPVs) and hybrids have been observed in Brazil, India, USA, South Africa and Zimbabwe; therefore development of cultivars with adequate levels of resistance to PLS should be possible. However, PLS resistance which is available in the important tropical inbreds that dominate hybrid parentage in tropical Africa has not been studied.

Resistance to PLS, GLS, NLB, and common rust diseases was shown to be quantitatively or polygenically inherited. However contradictory information exists as to whether the resistance is completely or partially dominant, as well as to the number of genes involved in the inheritance. From the different studies reported in literature, the additive-dominance model was adequate in explaining PLS and GLS disease resistance; however, others have suggested existence of non-allelic interactions, thereby making the model inadequate. This has an effect on the breeding strategy that can be used. The suggestion of the possible role of maternal effects in influencing resistance to PLS and GLS needs investigation. This is because presence of maternal effects impacts negatively on the response to selection.

A significant GEI means breeding for specific adaptation as selections from one environment may perform poorly in another. Multi-location trials are therefore needed to determine if GEI exist. Yield stability remains an important factor in the highly variable African production environments. For diseases, costs can be cut by screening in one location, that is, if the GEI is not of the cross-over type. It is important therefore to investigate the type of GEI for PLS, GLS, NLB and common rust in the African adapted germplasm

Although most of the studies reported in literature have provided useful genetic information for the various diseases, most of them involved temperate inbreds which were tested in the temperate environments. In addition application of the genetic information is usually restricted to the base germplasm used in the specific studies, as most of them involved fixed models for the genotypes. This therefore justifies the need to investigate the gene action and combining ability for the germplasm that is adapted to the tropical African germplasm.

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2 Identification of Farmers' Key Maize Production Constraints and Traits Desired in Maize Cultivars

Abstract

Maize is the staple food crop for the majority of households in Southern Africa. However, yields in the smallholder (SH) farming sector in the region have remained low, despite the availability of many high yielding improved varieties including hybrids. The objectives of this study were therefore to establish the preferred maize characteristics by SH farmers and to identify and analyse the constraints to maize production in a selected smallholder (SH) farming area of KwaZulu-Natal (KZN) Province in South Africa. Structured surveys of 300 households and participatory rural appraisal (PRA) involving focus group discussion were conducted between January – May 2007 in Obonjaneni, Busingatha and Okhombe villages in the Northern Drakensberg. The PRA methodologies used included problem listing, analysis, matrix scoring and pairwise ranking of traits. Samples of local varieties were evaluated under researcher managed trials for disease resistance and grain yield potential at Cedara Agricultural Research Station and Baynesfield Estate. The PRA and structured surveys established that maize was the principal crop grown in the three villages. One hundred percent of the farmers grew the local landrace they called *Natal-8-row* or *IsiZulu*. Hybrids and improved open pollinated varieties (OPVs) were planted by less than 40% of the farmers. The farmers preferred the local landrace for its taste, recycled seed, tolerance to abiotic stresses and yield stability. Preferred characteristics of maize varieties were inexpensive seed, high yield, early maturity and low input costs. Taste was not ranked highly, although it was amongst the top perceived advantages of the local variety. Pests/diseases and drought were not ranked highly, as the farmers indicated that they planted early to escape diseases and drought. The local varieties had high yield potential and exhibited genetic variability for disease resistance that can be exploited in breeding programmes. Farmers indicated they were willing to grow hybrids if the cost of seed and other inputs were affordable. Abiotic stresses were amongst the top four constraints faced by the farmers, whereas, biotic stresses were not ranked that highly. The results showed that if the main production constraints were addressed, farmers could realize high yields from their local varieties. Breeding opportunities therefore exist for incorporating tolerance to abiotic stresses in the local varieties.

2.1 Introduction

Production of maize in Sub Saharan Africa (SSA) is dominated by small-scale farmers who have land holdings ranging from 0.5 to 3.0 ha (Byerlee and Helsey, 1997). The majority of these farmers are located in marginal areas which are highly variable and stress-prone and they depend, therefore, on extremely low-input, low-risk cropping systems (Shumba, 1984; Banziger and de Meyer, 2002; Reeves and Cassaday, 2002). Consequently, maize yields in these areas have remained low, averaging below 1.2 t ha^{-1} against a potential of 7.0 t ha^{-1} (Pingali and Pandey, 2001). Although improved, superior cultivars have been developed in most of the countries in SSA, the majority of the small-scale farmers still rely on unimproved open-pollinated varieties (OPVs) for their plantings (FAO and CIMMYT, 1997; Aquino *et al*., 2001). This is partly because the OPVs are easy to multiply and therefore cheap and readily available (FAO and CIMMYT, 1997). In addition, however, most of the breeders of improved cultivars have focused more on raising yields under optimal, agronomically well-managed conditions (Reeves and Cassaday, 2002) and farmers either perceive little advantage in growing them because they are not designed for their needs (Banziger and Diallo, 2002).

Breeders have often been accused of failing to consider the special preferences of farmers especially those in marginal areas (Toomey, 1999; Banziger and Cooper, 2001), possibly because they are unaware of them. Therefore, for effective breeding, farmers' perceived constraints and their preferences for cultivars should be clearly identified through researcher-farmer interaction and collaboration. Farmer participatory research has been defined as the "*collaboration of farmers and scientists in agricultural research and development*" (Bentley, 1994). Participatory methods are now preferred as they recognize the value of farmers' local knowledge, their interests and ability to experiment and innovate, and their active exchange of information and technologies as well as the fact that the farmers are not a homogeneous group — they have different preferences and priorities (Bellon, 2001).

Farmers can provide very important information on plant types, desired traits and insight into trade-offs they are willing to make among traits in designing cultivar types (Sperling *et al*., 2001). For example, farmers in eastern Kenya indicated preference for early maturity ahead of yield followed by yield-related traits namely cob size, grain size and drought tolerance (de Groote *et al*., 2000). In Southern Africa, Banziger and de Meyer (2002) reported that apart from yield related traits, farmers frequently mention early maturing varieties, hard endosperm (flint) types and good husk cover for the maize

varieties they would prefer. A study conducted in the Guinea savannas of Nigeria also indicated differences by farmers in their preferred choice of maize varieties (Kamara *et al*., 2006). For example, farmers from the relatively market-driven production systems in the communities of Borno State, Nigeria preferred the early-maturing and high-yielding drought-tolerant varieties (Kamara et al., 2006). In contrast, farmers from the relatively resource-poor sorghum-based production systems in Kano State, Nigeria preferred extra-early maturing varieties to provide food security during the period of food scarcity rather than high yielding varieties (Kamara *et al*., 2006). In Ethiopia, studies by Abebe *et al.* (2005) also revealed that, generally farmers have their own way of selecting a variety for their localities, although in some cases the farmers' preferences coincide with the breeders' selection. It is, therefore, important to determine from farmers their preferred traits in crop varieties or include the farmers in a variety selection process. This enhances the potential for adoption of the varieties in the respective communities where the studies are conducted.

The objectives of this study were therefore to i) establish the preferred maize characteristics by smallholder (SH) farmers in selected districts of KwaZulu-Natal (KZN) Province, South Africa that can be used for selection in breeding programmes, and ii) identify and analyse the constraints to maize production in a selected SH farming area of KZN.

2.2 Research methodology

2.2.1 Study area

The study was conducted in three villages of Obonjaneni, Busingatha and Okombe in Amazizi Tribal Authority in the Northern Drankensberg of KwaZulu-Natal (KZN) Province during 2007 growing season. The area is rural but near the Royal Natal National Park and a lot of other resort areas. It therefore has strong links to tarred roads which provide access for tourists who come to the different resorts (Krone, 2006). The area was chosen purposely as the farmers have been growing maize every season and it is a research area for Farmer Support Group based at UKZN, and therefore a lot of secondary data were available, which were analysed for this particular study. The population in the villages is approximately 900 households in Obonjaneni, 700 in Busingatha and 1,000 in Okhombe, giving a total of 2,600 households (Krone, 2006). The area is characterised by an average annual rainfall between 700-800 mm (Ngubane and Mudhara, 2009). Thunderstorms and intermittent dry spells are a common characteristic of the rainfall

pattern. The rainy season normally lasts from September/October to March (Ngubane and Mudhara, 2009). Soils in the area are acidic. However, the area is classified as having above average agricultural potential. The major drawback, however, is the short growing season; cold winters and acidic soils which tend to reduce the agricultural potential (Krone, 2006).

2.2.2 Sampling procedures and participants

Three hundred households were included in the structured survey and these were selected randomly. The breakdown of the farmers who participated based on gender is presented in Table 2.1. More females than males from each village participated in the structured survey. Overall, across the villages, 59% of the respondents were females. In addition there were five focus group discussions of ± 10 key informants that included individuals who had great knowledge about the villages, the farms, crops and local conditions and problems in the district. Selection of this group was done in consultation with the facilitator who resided in the area and had knowledge of the farmers around. The farmers selected were a mixed group of males and females, farmers who planted many crop varieties, farmers who had a reputation for good, workmanship, young and old farmers, and farmers with large or small land holdings. The research team comprised of the principal investigator, two facilitators with knowledge of the area and the local language, isiZulu. The extension personnel were busy during the research period and only one of them managed to attend a focus group discussion in Obonjaneni.

Table 2.1 Total number of farmers interviewed in structured survey and those who participated in focus group discussions in Amazizi.

Village	Male	Female	Total
Formal survey			
Obonjaneni	35	55	90
Busingatha	34	59	93
Okhombe	46	51	97
Total	**115 (41.1%)**	**165 (58.9%)**	**280**
Focus group discussion			
Obonjaneni	7	5	12
Busingatha	7	8	15
Okhombe	10	8	18
Total	**24 (53.3%)**	**21 (46.7%)**	**45**

Note: A total of 300 farmers were interviewed and 20 did not indicate their gender in structured surveys (Obonjaneni -10, Busingatha - 7, Okhombe – 3).

2.2.3 Data collection

Primary data were collected through a structured survey to obtain characteristics of the farmers in the districts and through participatory methodologies. The participatory methodologies used included focus group discussions for matrix scoring and pair wise ranking and walk with some of the farmers to the fields. Only two farmers' fields were visited as most of the maize had already dried and the farmers had harvested the crop. During the walk, the principal investigator encouraged discussion on what the farmers used the maize for, whether they had specific cultivars for the different uses, sources of maize seed, and problems they faced in maize production. The state of roads, water sources and condition of the fields were also noted. Notes were taken during the walk and samples of interesting seeds of local landraces and other maize varieties were collected with the farmers' permission.

2.2.4 Structured survey

The survey served as a control for checking or comparing information obtained through participatory methods. Information was gathered through a questionnaire administered to the farmers by the facilitators. The questionnaire was pre-tested on a small sample that included staff from FSG and farmers from the area and based on the responses from this survey trial, adjustments were made to the questionnaire. Characteristics that the farmers considered important in maize cultivars and the perceived constraints to maize production were obtained from this survey in addition to other general information.

2.2.5 Focus group discussions

To learn about the farmers' classifications and choices, matrix scoring and pair-wise ranking methods were used. The technique used consisted of problem listing, analysis and ranking by the different groups. The groups were mixtures of males and females as the farmers did not want to be separated into groups based on gender. The female farmers conformed that the presence of men was not going to hold them back from freely expressing themselves. The discussions were guided by two facilitators in the local language and points written down in English on a flip chart. Overall, 45 farmers participated in the focus group discussions. The numbers of male and female farmers who participated were almost similar, with 53% males and 47% females participating (Table 2.1). The gender composition in the focus group discussion was affected by apologies from a number of the women on the day of the discussions, and it therefore did not reflect the composition observed in the structured survey.

The first goal of the focus group discussions was to identify maize varieties and traits that were relevant for each type. The farmers listed the varieties they grew, ranked them, and identified traits they preferred in maize, giving reasons for the varieties they liked to continue growing. The second goal was to identify “core problems or constraints” to maize production. Farmers again identified the problems, listed them and ranked them according to the most important constraints. The facilitators used pictures showing disease symptoms and cards that had drawings representing various traits and constraints to assist the farmers during the discussions.

For pair-wise ranking, traits of interest were compared pair by pair; groups were asked which of the two they preferred, and why. In matrix scoring, the criteria were placed in rows in a matrix and the varieties in columns. The farmers were asked to complete the boxes row by row, giving a score for each of the characteristics. The scores used were 1=very poor to 5 = excellent.

2.2.6 Biotic stresses

Special reference was made on the prevalence of the main biotic stresses, such as pests, diseases and weeds. The farmers listed the diseases, pests and weeds that occurred in their area and then indicated which ones were problematic and difficult to control. They then pointed out whether they used any form of control or not and listed some of the control methods they used. To validate whether the local varieties grown were susceptible or resistant to some of the major diseases that occurred in KwaZulu-Natal (KZN), ten maize seed collections from the farmers were evaluated over two seasons at Cedara Agricultural Research Station (30°16’E, 29°32’S, 1130 metres above sea level (m.a.s.l)) and Baynesfield Estate (30°21’E, 29°46’S, 758 m.a.s.l) for yield and disease resistance potential in 2007/8 season at Cedara and 2008/9 season at Cedara and Baynesfield giving a total of three environments. These two sites are “hot spot areas” for a lot of maize diseases. The 10 collections together with an OPV standard check were planted in an 11 x 2 randomised block design (RCBD) with two replications per site in two row plots, 3 m long, with 0.75 m inter-row spacing and 0.3 m intra-row spacing. Plant population densities were about 44 000 per hectare in all the seasons. A Kenyan population, which is an OPV and popular with smallholder farmers in Kenya was used as a check for the trial. Two blocks of a susceptible maize hybrid (PAN 6017) were used as borders for the trials. Standard cultural practices including ploughing and disking, hand

planting, hand weeding and/or application of herbicides and fertilizers were followed at each site.

Severity for the foliar diseases was assessed from the first appearance of symptoms, based on visual assessment of the whole plot. A 1-9 logarithmic rating scale was used where 1 = 0%, 2 = <1%, 3 = 1-3%, 4 = 4-6%, 5 = 7-12%, 6 = 13-25%, 7 = 26-50%, 8 = 51-75% and 9 = 75-100% leaf area showing disease symptoms. The scores were further classified into the following disease reaction types; 1.0 = symptomless, 2.0-4.0 = resistant, 4.1-5.0 = moderately resistant, 5.1-6.0 = moderately susceptible, 6.1-9.0 = susceptible.

The local varieties were also self-pollinated over four cycles and plants selected that were resistant to major diseases. This was to develop maize lines with the characteristics that the farmers wanted, but also with resistance to the major diseases that are prevalent in the area.

2.3 Data analysis

Statistical analyses of both quantitative and qualitative data were performed in SPSS (Release 15.0) computer package, Genstat 12th edition (Payne *et al*., 2009) and PROC GLM procedure in SAS 9.1 (SAS Institute, 2002). Data were classified as nominal or ordinal when entering into the SPSS spreadsheet. For exploring relationships; frequencies, descriptive statistics and analysis of variance (ANOVA) were computed for data collected in each village followed by mean comparisons between villages. Chi-square test for association between qualitative variables was performed. Before subjecting the data to ANOVA, percentage data were transformed using $\log_{10}$ or square root transformation to normalize the data.

2.4 Results

2.4.1 General crop production aspects and uses of maize

The general aspects of crop production for the three villages are presented in Appendix 2.1. There were significant ($P<0.001$) differences amongst villages in terms of land holdings and the area of land allocated to maize cultivation. The average land size across the villages was 1.4 ha and of this, about 80 to 90% was used for cultivation. Of the land used for cultivation, 94 to 98% was allocated to maize production and the

remainder to other crops. Across the villages, maize was grown by 100% of the farmers who responded and was ranked first by the farmers who participated in the focus group discussions. The percentage of farmers growing other crops was less than 10%. In focus groups, vegetables were ranked second to maize, followed by potatoes, beans and others.

Maize was grown mainly for consumption. However, other farmers, in addition to consumption also grew maize for livestock feed and for sale. The average grain yield was significantly different (P=0.001) in the three villages ranging from 0.2 to 5.7 t ha^{-1}, with an average across the villages of 1.0 t ha^{-1}.

Maize was used in a variety of products. The most important was mealie-meal which was used for the traditional meal, a thick porridge – *puthu* or *pap* consumed with vegetables and/or meat. Other uses included breakfast porridge (both white and yellow maize was used), roasted or boiled green mealies (both yellow and white maize) and samp (mealie-rice). Yellow maize was also used for the traditional beer *mtombo*. An average of 46% of the farmers used yellow maize for livestock feed as well.

2.4.2 Varieties of maize grown and sources of seed in Amazizi district

Different varieties which included hybrids, open-pollinated varieties (OPVs) and local landraces were grown by the farmers. These varieties are presented in Table 2.2. The majority of the farmers grew the local or indigenous variety (landrace) which they called *Natal-8-row* or *IsiZulu*. The name came from the number of rows which was eight in most of the cases. This variety is an open pollinated variety (OPV) and the farmers recycled the seed or obtained from other farmers. Collections of this variety are presented in Fig. 2.1. The variety was highly variable in colour ranging from white, yellow to mixtures. There were also some variants which were white, red and maroon mosaics. The different cobs collected from the farmers were characterized by the principal investigator based on the kernel size, the number of rows per cob, grain texture, husk cover and ear aspect. Collections of the other local variety *Doylanda* and the improved OPV Kalahari Early Pearl (KEP) are presented in Fig. 2.1. The farmers indicated that *Doylanda* (DL) was a hybrid between the local *Natal-8-row* (NTL8) and Pannar (PAN) hybrids that were grown in the area. However, the seed of DL was also recycled over a number of seasons. The major difference with the NTL8 was that DL had more than eight rows.

The most popular hybrids were PAN hybrids. These were grown by a small percentage of the farmers (18 to 39%). Most of the farmers who grew PAN hybrids had large landholdings and also sold part of the maize produced. Other improved OPVs were grown by an even smaller percentage of farmers and these are indicated in Table 2.2. Most of these OPVs were available from the Input shops.

Sources of seed of the varieties grown by the farmers are presented in Table 2.3. The farmers indicated that for the OPVs and local varieties (NTL8 and DL), they recycled the seed and in a few cases they got it from other farmers. The farmers also pointed out that the local varieties were, however, susceptible to weevils if kept for more than 2 years without treatment. A small percentage of the farmers indicated that they saved hybrid seed.

Table 2.2 Varieties mentioned and percentage of farmers growing them in Amazizi district

	Formal Survey			Focus groups					
Variety†	OBO‡	BUS	OKH	OBO	BUS	OKH	Colour	Type	Source of seed
unknown yellow	1	1	5	-	-	-	yellow	local?	Farm saved
unknown white	-	-	1	-	-	-	white	local?	Farm saved
IsiZulu (Natal-8-row)	77	90	97	100	100	100	white, yellow, mixture	local[1]	Farm saved Other farmers
PAN 6479	8	7	-	33	27	39	white	hybrid[2]	Input shop
PAN 6043	-	-	-	33	-	13	white	hybrid	Input shop
PAN 6480	7	5	5	33	27	39	yellow	hybrid	Input shop
PAN 6825	-	-	-	-	-	13	white	hybrid	Input shop
Pannar brand	18	1	-	-	-	-	white or yellow	hybrid	Input shop
Doylanda	2	-	3	-	-	-	white, yellow, mixture	local	Farm saved
R0413	2	1	-	-	-	-		OPV[3]	Input shop Farm saved
Kalahari Early Pearl	-	-	4	-	-	22	white	OPV	NGO
Nelson's choice	1	-	-	-	-	-	white	OPV	Input shop Farm saved
Afric1	-	-	1	-	-	-	white	OPV	Input shop Farm saved

†*IsiZulu* or *Natal-8-row* - local or indigenous variety, *Doylanda* – a variant from Natal-8-row, which was a hybrid between Natal-8-row and some Pannar varieties that were grown in the area.
‡OBO = Obonjaneni, BUS = Busingatha, OKH = Okhombe
[1]local – no specific name, but planted by the farmers in the community for many years, [2]hybrid – name provided by the farmers of a known hybrid or a company that sells hybrids, [3]OPV – name provided by the farmers of a known OPV whose seed was bought from the shop, then recycled over a number of seasons.

Table 2.3 Farmers' Source of maize seed (%)

	Village								
	Obonjaneni			Busingatha			Okhombe		
	Local† (n=78)	Hybrids (n=38)	OPVs (n=5)	Local (n=90)	Hybrids (n=20)	OPVs (n=5)	Local (n=97)	Hybrids (n=10)	OPVs (n=12)
Farm saved	79.5	16.0	-	86.6	-	20	90.7	-	58.3
Input shop	1.3	81.6	80	2.2	60	20	-	50	16.7
Other farmers	18	2.6	-	11.1	-	-	9.3	-	-
NGO‡	-	-	-	-	-	-	-	-	33.3

†Local = *Natal-8-row* and *Doylanda*, Hybrids = Pannar hybrids, OPVs = Afric1, Kalahari Early Pearl, Nelson's choice and R0413, ‡NGO = Non-Governmental Organisations

Figure 2.1 Collections of maize from local farmers

The farmers' harvest in Fig 2.1b was predominantly yellow, although there were some cobs which were more on the orange side. The farmer indicated that the yellow maize was used mainly for livestock feed. The NTL8 and DL (Fig 2.1c and d) were characterized by large kernels, 8-12 rows, with variable grain colour (white, yellow, mixtures of white, yellow red and maroon). Most of the cobs were clean, with no cob rots

and the grain texture ranged from flint to dent. In general the ear aspect was good, but the cob size was variable as indicated in Fig 2.1c and d. Kalahari Early Pearl was characterized by large cobs (Fig 2.1e) but small kernels. The grain was predominantly white and the texture dent. The cobs were clean with no cob rots and had 16 rows each.

2.4.3 Differentiation of maize varieties by the farmers

Farmers had their own attributes they used to differentiate the maize varieties that were grown in the area. These attributes are presented in Table 2.4. Most of the farmers used kernel size and number of rows per cob to differentiate the varieties grown. Ninety-three percent and 47% of the farmers identified NTL8 as having big kernels and 8-12 rows, respectively, whereas the hybrids and improved OPVs had smaller kernels as indicated by more than 80% of the farmers. Twenty-three to 31% of the farmers identified the hybrids and OPVS as having more than 12 rows per cob.

Table 2.4 Attributes used by farmers to differentiate the maize varieties grown in Amazizi district and percentage of farmers responding

	Village											
	OBO‡			BUS			OKH			Overall Mean		
	Local†	Hybrids	OPVs	Local	Hybrids	OPVs	Local	Hybrids	OPVs	Local	Hybrids	OPVs
8-12 rows	19.0	-	-	50.0	-	-	55.0	-	-	47.0	-	-
>12 rows	1.0	7.0	1.0	-	3.0	-	-	2.0	4.0	0.408	23.5	31.3
Large kernels	71.0	-	1.0	85.0	-	-	89.0	-	2.0	92.8	-	18.8
Small kernels	3.0	25.0	5.0	2.0	11.0	1.0	-	5.0	8.0	1.9	80.4	87.5
White	1.0	5.0	-	8.0	4.0	-	5.0	-	2.0	5.3	17.7	12.5
Yellow	-	4.0	1.0	-	1.0	-	-	1.0	3.0	-	11.8	25.0
Mixed	1.0	-	-	1.0	-	-	-	-	-	0.8	-	-
Seed is treated	-	4.0	-	-	-	-	-	-	-	-	7.8	-
Early maturity	1.0	-	-	1.0	-	-	2.0	-	-	1.5	-	-
Shorter stalks	-	-	-	2.0	-	-	1.0	-	-	1.1	-	-
Turned down leaves	7.0	-	-	4.0	-	-	1.0	-	-	4.6	-	-
Leaves turned upwards	-	2.0	-	-	-	-	-	-	-	-	3.9	-
Broad shaped leaves	1.0	1.0	-	-	1.0	-	-	-	-	0.4	3.9	-
Narrow shaped leaves	-	3.0	-	-	-	-	-	-	-	-	5.9	-
Hard flat kernels	2.0	-	-	-	-	-	2.0	-	-	1.5	-	-
Dark in colour	-	-	-	1.0	-	-	1.0	-	-	0.8	-	-
Long stalks	-	-	-	-	1.0	-	-	-	-	-	2.0	-

†Local = *Natal-8-row* and *Doylanda*, Hybrids = Pannar hybrids, OPVs = Afric1, Kalahari Early Pearl, Nelson's choice and R0413, ‡OBO = Obonjaneni, BUS = Busingatha, OKH = Okhombe. Groupings "local", "hybrid" and "OPVs" were used for presenting the results as the farmers gave similar responses for differentiating the cultivars within each of the groups.

2.4.4 Farmers perceived advantages and disadvantages of the different maize types

The farmers indicated why they preferred the varieties they grew. Their responses are presented in Table 2.5. Ninety-three percent of the farmers from the structured survey indicated that they preferred NTL8 and DL. This was mainly because they were tasty and less expensive since the farmers could save their own seed or obtain it from other farmers at no cost. They also pointed out that they could grow the varieties with manure only and still get a satisfactory yield. The major disadvantage was that it was affected by diseases and insects, notably weevils which affected untreated seed in storage, especially when kept for more than two years. In addition 14.6% of the farmers mentioned that the local varieties gave low yields.

The hybrids were preferred mainly because of high yield and disease resistance, but the majority of farmers indicated that they were expensive to grow as they could not save the seed and they required fertilizers always. Thirty-four percent of the farmers also pointed out that, although the mealie-meal was white, it was not tasty. Others preferred the hybrids because of the number of cobs per plant, which varied from 2-3 cobs, more than 12 rows per cob, plus the hybrids were quick to dry and easy to shell.

The yellow maize was preferred mainly for livestock feed and consumption as roasted mealies or porridge. In addition, the seed could also be recycled. The improved OPVs, on the other hand, were favoured mainly for high yield compared to the local varieties. Thirty percent of the farmers also indicated that the OPVs were disease resistant, easy to shell and had many rows (14-20) per cob. However, the downside was that they were affected by drought and the mealie-meal was not tasty.

Table 2.5 Farmers' perceived advantages and disadvantages of the different maize types grown in their area, Amazizi and the percentage of farmers mentioning the trait.

	Maize type							
	Local †		Hybrids		Yellow maize (Unknown brand)		OPVs	
	(n = 280)	%	(n = 35)	%	(n = 10)	%	(n = 10)	%
Advantages	Tasty in all foods	93.5	High yield	74.3	Good for both livestock and consumption	60.0	High yield	50.0
	Save seed	60.7	Disease resistant	42.9	High yield	10.0	Save seed	30.0
	Sweet	44.6	Insect resistant	17.1	Save seed	50.0	Disease resistant	30.0
	Inexpensive variety	16.1	Mealie-meal white	20.0	No fertilizer needed	10.0	14-20 rows/cob	10.0
	Early maturity	13.9	2-3 cobs/plant	14.3	Disease resistant	10.0	Early maturity	10.0
	Enough/satisfactory yield	11.1	14-20 rows/cob	14.3			Easy to shell	20.0
	Drought tolerant	5.36	Easy to shell	20.0			Insect resistant	10.0
	Use manure only	8.6	Withstand lodging	17.1			2 cobs/plant	20.0
	No fertilizer or manure	4.0	Quick to dry	14.3				
	Cob rot resistant	2.5						
	Withstand lodging	2.1						
	Large kernels	5.4						
Disadvantages	Affected by diseases	39.3	Cannot save seed	42.9	Not suitable for all foods	30.0	Affected by drought	20.0
	Low yield	14.6	Late maturity	28.6	Affected by diseases	20.0	Small kernels	30.0
	Affected by weevils	15.7	Not tasty	34.3	Affected by insects	20.0	Mealie-meal not tasty	20.0
	Affected by insects	26.8	Expensive variety	22.9				
	Mealie-meal dark	5.7	Affected by drought	14.3				
	Takes long to dry	5.4	Need to apply fertilizer	17.1				
	Hard to grind	5.7	Small kernels	11.4				
	Affected by stalkborer	5.6						
	Affected by cutworm	3.2						

†Local = *Natal-8-row* and *Doylanda*, Hybrids = Pannar hybrids, OPVs = Afric1, Kalahari Early Pearl, Nelson's choice and R0413.
Groupings "local", "hybrid" and "OPVs" were used for presenting the results as the farmers gave similar responses for the cultivars within each of the groups.

2.4.5 Farmers ranking of their varieties according to their own criteria

Results from focus group discussion of the pair-wise ranking of the characteristics preferred by farmers are presented in Table 2.6.

Table 2.6 Pair-wise ranking of the characteristics preferred by the farmers in Amazizi district during focus group discussions

	Characteristic†	A	B	C	D	E	F	G	H	I	J	K	L	Score‡	Rank
Obonjaneni															
A	Seed easy to get	-	A	A	A	A	A	A	A	A	A	A		**10**	**1**
B	Taste		-	C	D	B	F	B	B	B	J	K		**4**	**7**
C	Yield			-	C	C	F	C	C	C	C	C		**8**	**2**
D	Pest/disease resistant				-	D	F	D	D	D	J	D		**6**	**4**
E	Drought resistant					-	F	E	E	E	E	E		**5**	**5**
F	Early maturity						-	F	F	F	J	F		**8**	**2**
G	Good for sale							-	G	I	J	J		**1**	**9**
H	Good for livestock								-	H	J	K		**1**	**9**
I	Consumed in a variety of foods									-	J	I		**2**	**8**
J	More lines per cob										-	K		**7**	**3**
K	More than one cob											-		**3**	**6**
Busingatha															
A	Seed easy to get	-	A	C	A	A	A	A	A	A	A	A	A	**10**	**2**
B	Taste		-	C	D	E	F	B	B	B	J	B	L	**4**	**5**
C	Yield			-	C	C	C	C	C	C	C	C	C	**11**	**1**
D	Pest/disease resistant				-	D	F	D	D	D	D	D	L	**7**	**3**
E	drought resistant					-	E	F	E	E	E	E	E	**7**	**3**
F	Early maturity						-	F	F	F	J	F	L	**7**	**3**
G	Good for sale							-	G	I	J	K	L	**1**	**8**
H	Good for livestock								-	H	J	K	L	**1**	**8**
I	Consumed in a variety of foods									-	I	K	L	**2**	**7**
J	More lines per cob										-	J	J	**6**	**4**
K	More than one cob											-	L	**3**	**6**
L	Less inputs needed												-	**7**	**3**
Okhombe															
A	Seed easy to get	-	A	A	A	A	A	A	A	A	A	A	A	**11**	**1**
B	Taste		-	C	B	B	F	B	B	B	B	B	L	**7**	**3**
C	Yield			-	C	C	C	C	C	C	C	C	C	**10**	**2**
D	Pest/disease resistant				-	E	D	D	D	D	J	D	L	**5**	**5**
E	drought resistant					-	E	E	E	E	E	E	L	**7**	**3**
F	Early maturity						-	F	F	F	J	F	F	**6**	**4**
G	Good for sale							-	H	G	G	K	L	**2**	**7**
H	Good for livestock								-	H	J	H	H	**4**	**6**
I	Consumed in a variety of foods									-	I	K	I	**2**	**7**
J	More lines per cob										-	J	L	**4**	**6**
K	More than one cob											-	L	**2**	**7**
L	Less inputs needed												-	**6**	**4**

†The characteristics for the columns are indicated by letters, which correspond to the letters of the listed trait. ‡ The score was equivalent to the frequency of the letter in the row representing the characteristic. Low score = high rank and indicates that the characteristic is less important.

The most important criteria considered by farmers in Obonjaneni were the acquisition of seed, yield and early maturity, followed by more rows on a cob, pest/diseases resistant and drought (Table 2.6). In Obonjaneni, the farmers did not include on their list of characteristics, the input requirement. In Busingatha, the preferred criteria in order of importance were yield, seed procurement, followed by pest/diseases, drought, early in maturity and less inputs needed. Taste was not an important criterion for the farmers from Obonjaneni and Busingatha. Farmers from Okhombe rated seed procurement and high yield as the most important criteria in maize varieties, followed by taste and drought resistance.

The overall score and rank from these group discussions are presented in Table 2.7. Across the villages, the preferred characteristics were ease to get seed, yield, and early in maturity. The three characteristics were not significantly different from each other in importance. Low inputs, pest/disease resistant, drought resistant, more lines per cob and taste were not significantly different from each other. The last four characteristics which included whether the variety was good for livestock or sale, consumption in a variety of foods were not significantly different from each other and were ranked last by the farmers.

Table 2.7 Pairwise ranking of the characteristics preferred by the farmers in Amazizi district during focus group discussions across the three villages

	Score†			Overall Mean	
Characteristic	Obonjaneni	Busingatha	Okhombe	**Score**	**Rank‡**
Seed easy to get	10	10	11	10.3[a]	1
Yield	8	11	10	9.7[a]	1
Early maturity	8	7	6	7.0[a]	1
Less inputs needed	§-	7	6	6.5[b]	2
drought resistant	5	7	7	6.3[b]	2
Pest/disease resistant	6	7	5	6.0[b]	2
More lines per cob	7	6	4	5.7[b]	2
Taste	4	4	7	5.0[b]	2
More than one cob	3	3	2	2.7[c]	3
Good for livestock	1	1	4	2.0[c]	3
Consumed in a variety of foods	2	2	2	2.0[c]	3
Good for sale	1	1	2	1.3[c]	3
Mean				5.3	
LSD(0.05)				2.1	
CV (%)				21.9	

†, Score from Table 2.6. ‡Low score = high rank and indicates that the characteristic is less important. §– = not a criterion in the area. Means followed by the same letter are not significantly different and therefore the characteristics were ranked the same.

The important characteristics were also obtained through the structured survey. The farmers who participated in the structured survey came up with their own characteristics they considered were important for an "ideal" variety. These are presented in Table 2.8. Some of the characteristics were similar to what farmers listed in group discussions. There were a few additional ones which included tolerance to acidity, resistance to lodging and some specifics like weevil resistance.

Table 2.8 Characteristics of an "Ideal" variety as indicated by farmers during the structured survey (% of farmers selecting characteristic)

	Village			Mean	Rank‡
	Obonjaneni	Busingatha	Okhombe		
	%	%	%		
High yield	6.2 (38.0)†	3.7 (14.0)	4.2 (18.0)	5.1 (26.0)	1
Good taste	4.6 (21.0)	2.2 (5.0)	3.3 (11.0)	4.9 (23.7)	2
Low input variety	3.3 (11.0)	3.0 (9.0)	3.0 (9.0)	4.6 (21.0)	3
Inexpensive seed	4.9 (24.0)	3.0 (9.0)	3.7 (14.0)	4.6 (22.3)	3
Early maturing	2.4 (6.0)	2.4 (6.0)	1.7 (3.0)	4.0 (16.3)	4
Disease resistant	5.5 (30.0)	2.6 (7.0)	2.8 (8.0)	4.0 (17.7)	4
Tolerant to acid soils	3.0 (9.0)	1.7 (3.0)	1.7 (3.0)	4.0 (16.3)	4
Drought resistant	3.2 (10.0)	1.4 (2.0)	2.2 (5.0)	2.9 (8.3)	5
Enough/satisfactory yield	2.4 (6.0)	1.7 (3.0)	2.0 (4.0)	2.1 (4.3)	5
2-3 cobs	1.0 (1.0)	2.0 (4.0)	2.2 (5.0)	1.8 (3.3)	6
Insect resistant	4.6 (21.0)	0.0 (0.0)	0.0 (0.0)	1.5 (10.5)	7
All purpose variety	2.2 (5.0)	0.0 (0.0)	1.7 (3.0)	1.3 (4.0)	7
Resistant to lodging	2.0 (4.0)	0.0 (0.0)	1.4 (2.0)	1.1 (3.0)	7
Weevil resistance	1.4 (2.0)	0.0 (0.0)	1.7 (3.0)	1.1 (2.5)	7
Good cooking qualities	2.8 (8.0)	0.0 (0.0)	0.0 (0.0)	0.9 (4.0)	7
Mean				2.9	
P-value				<.001	
S.e.d				0.97	
Lsd(0.05)				1.42	

†Data transformed (square root transformation). Values in parenthesis are the untransformed percentages.
‡Ranking based on transformed means. The lower the rank, the more important the constraint

The farmers from the different villages had different characteristics for an "ideal" variety. Farmers from Obonjaneni listed the following in order of importance; high yield, disease resistance, inexpensive seed, insect resistance and good taste as the most important. However, this was different from Busingatha where the farmers considered; high yield, inexpensive seed and low inputs, disease resistance and early maturity amongst the most important attributes. Farmers from Okhombe had high yield, inexpensive seed, good taste, low inputs and disease resistance amongst the important characteristics. On the whole, the characteristics that were ranked between 1 and 4 were; high yield (1),

followed by good taste (2), inexpensive seed and low inputs (3), early maturity, disease resistance and tolerance to acid soils (4).

2.4.6 Farmers evaluation of the main maize varieties grown in Amazizi district through matrix ranking

Results of matrix scoring for the main four varieties of maize grown in Amazizi district are presented in Table 2.9. Four varieties; NTL8, PAN hybrids, KEP and Afric1 were compared with the characteristics listed by the farmers during the focus group discussions. The scores for each variety were added and the mean calculated and used to rank the maize varieties.

Table 2.9 Farmers' evaluation of their varieties according to their own criteria (1=very poor, 5 = excellent)

	Obonjaneni				Busingatha				Okhombe			
	NTL8†	PAN	KEP	Afric1	NTL8	PAN	KEP	Afric1	NTL8	PAN	KEP	Afric1
Save seed	5	1	5	5	5	1	5	5	5	1	5	5
Early maturity	5	2	4	4	4	3	4	4	4	3	4	4
Yield	3	5	5	4	3	5	5	4	2	5	5	4
Number of lines/cob	1	5	5	5	1	5	5	5	1	5	5	5
Number of cobs/plant	1	5	5	5	1	5	5	5	1	5	5	5
Grain size	5	2	2	3	5	2	2	3	5	2	2	2
Cob rots	5	3	4	5	5	4	4	5	5	4	4	4
Tolerant to diseases	3	5	3	5	3	5	3	4	3	5	4	4
Insect resistance	2	4	4	4	2	4	4	4	3	4	4	5
Drought tolerant	4	3	4	4	3	3	3	3	3	3	4	3
Withstand lodging	4	3	3	3	5	4	4	4	5	3	4	3
Tolerant to low N	5	1	3	3	4	1	3	3	4	2	3	3
Tolerant to acid soils	4	1	2	2	4	1	3	2	3	1	2	2
Taste	5	2	4	4	5	2	3	4	5	3	4	4
Colour of mealie-meal	2	5	4	4	2	5	4	4	2	5	4	4
Good for sale	2	5	3	3	2	5	4	4	2	5	3	2
Easy to shell	5	5	5	5	5	5	5	5	4	5	5	5
Easy to grind	2	5	5	5	3	5	4	4	3	5	5	5
Quick to dry	5	5	4	4	4	5	4	4	4	5	4	4
Total score	68	67	74	77	66	70	74	76	64	71	76	73
Mean	**3.6**	**3.5**	**3.9**	**4.1**	**3.5**	**3.7**	**3.9**	**4.00**	**3.4**	**3.7**	**4.0**	**3.8**
Overall scores and ranking												
Natal	198	4										
Pannar	208	3										
Kalahari	224	2										
Afric1	226	1										

†NTL8 = *Natal-8-row*, PAN = Pannar hybrids, KEP = Kalahari early pearl

Natal-8-row was rated highly by the farmers on aspects such as tolerance to low nitrogen (N) and acid soils, grain size, resistance to cob rots and taste. The farmers rated the variety poorly on aspects such as the number of rows, the number of cobs per plant, the dark colour of mealie-meal, difficult to shell and grind. The hybrids were rated highly on yield, number of cobs per plant, number of rows per cob, good for sale and white mealie-meal and that they were easy to shell and grind. The characteristics of hybrids that received low scores were not being able to save seed, and not tolerant to acid soils or low N. The two OPVs, KEP and Afric1 were both rated highly on the ease to get seed, having more than two cobs per plant, ease of shelling and grinding and tolerance to diseases. However, they got low scores on not good for sale and small grain size. Overall, Afric1 was selected as the best variety by the farmers, followed closely by KEP, PAN hybrids and last the local NTL8.

2.4.7 Important biotic stresses in Amazizi district

Farmers through the structured survey gave a list of the problem pests, diseases and weeds that were important in their area and these are listed in Table 2.10 and Appendix 2.2. During focus groups the farmers also listed the diseases that occurred in their areas and these are presented in Table 2.10. From the structured survey more than 70% of the farmers indicated that stalkborer and cutworms were the most prevalent pests in Amazizi district. Twenty-seven percent of the farmers indicated that although the two pests were prevalent, they were not a problem. However 72% and 54% of the farmers singled out the two as the problematic pests in the area. Only 1% of the farmers mentioned a disease with yellowish leaves as being problematic.

During focus group discussions, with the aid of pictures showing disease symptoms, the farmers listed the diseases that occurred in their areas. Farmers in Obonjaneni indicated cob rots, Phaeosphaeria leaf spot (PLS) and northern corn leaf blight (NLB) as the diseases that often affected their crops and the two pests; stalkborer and cutworms. In Busingatha, stalkborer was mentioned again as the most problematic pest and the diseases listed were grey leaf spot (GLS), maize streak virus (MSV) and NLB. In Okhombe farmers listed PLS, stalkborer, NLB and rust. The farmers indicated that, although they had observed these diseases, they were not a big problem in the area as their incidences were low and their occurrences infrequent.

Table 2.10 List of problem diseases, pests and weeds (% farmers responding)

	Structured Survey				Focus Group discussion		
	OBO†	BUS	OKH	Mean	OBO	BUS	OKH
Prevalent Diseases and pests							
Yellowish leaves	1.0	-	-	1.0	Cob rots	Stalkborer	PLS
Stalkborer	71.0	74.0	78.0	74.3	PLS‡	GLS	Stalkborer
Cutworms	80.0	67.0	68.0	71.7	NLB	MSV	NLB
Other§	46.0	40.0	46.0	44.0	Stalkborer	NLB	Rust
					Cutworms	Weevils	
						Cutworms	
Problem diseases and pests							
None	30.0	29.0	23.0	27.3			
Stalkborer	69.0	71.0	77.0	72.3			
Cutworms	69.0	53.0	42.0	54.7			
Weevils	6.0	2.0	-	4.0			
Other	10.0	18.0	25.0	17.7			

†OBO = Obonjaneni, BUS = Busingatha, OKH = Okhombe, ‡PLS = Phaeosphaeria leaf spot, NLB = Northern corn leaf blight, GLS = Grey leaf spot, MSV = Maize streak virus, §Other: the list of other pests and diseases are listed in Appendix 2.

Although, stalkborer and cutworms were a major problem in the area, 23 to 30% of the farmers in the three villages did not apply any chemicals to control them. For those who did control the pests, some of them used unknown chemicals. The complete list of all the control options used by the farmers is shown in Appendix 2.3.

2.4.8 Evaluation of farmers' maize collections for disease resistance and yield potential

Results of the disease screening and grain yield potential of the farmers' collections are presented in Table 2.11. The genotypes and environments were all significant ($P \leq 0.001$) for PLS, GLS and NLB diseases. For rust only the environments were significantly different ($P<0.001$), but the genotypes were not. The yield for all the genotypes were not significantly different ($P>0.05$).

Table 2.11 Combined Analysis of variance for diseases and yield (t ha^{-1}) for the farmers' collection from Amazizi evaluated at Cedara and Baynesfield Estate in 2007/8 and 2008/9 seasons

		PLS	GLS	NLB	Common Rust	Yield (t ha$^{-1)}$
Source	DF	MS	MS	MS	MS	MS
Environment (Env)	2	178.95***	142.38***	221.88***	7.68***	2.46
Rep(Env)	3	1.59**	10.62**	1.82	0.32	1.55
Genotype	10	8.07***	13.78***	4.10***	0.21	4.20
Genotype*Env	20	3.79***	2.66	2.96***	0.40	2.78
Error	30	0.32	1.95	0.92	0.72	2.05

*, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001, respectively. PLS = Phaeosphaeria leaf spot, GLS = Grey leaf spot, NLB = Northern corn leaf blight,

Means for diseases and grain yield for the different genotypes are indicated in Table 2.12. Mean comparisons were not done for common rust since there were no significant differences among the genotypes. The KEP variety was the most susceptible to PLS with scores ranging from 7.5 to 7.6, followed by NTL8 which had scores ranging from 5.3 to 6.3. The DL variety was moderately susceptible to resistant with scores ranging from 4.7 to 5.7. The Kenyan population had a resistant score of 4.3. Reactions to GLS were also variable among the genotypes. The DL variety had scores ranging from 6.5 to 7.7 and was the most susceptible, followed by NTL8 which had scores from 6.7 to 7.0. The KEP variety was the most resistant to GLS with scores from 3.2 to 4.5. The Kenyan population was moderately susceptible with a score of 5.5. Scores of NLB ranged from resistant to moderately susceptible. The KEP variety was moderately susceptible to NLB, whereas the other genotypes were resistant to moderately resistant with scores ranging from 3.2 to 4.7. The Kenyan population had the highest yield of 6.6 t ha^{-1}, whereas yield for KEP variety varied from 4.8 to 6.4 t ha^{-1}, NTL8 from 5.3 to 6.3 t ha^{-1} and DL from 3.9 to 6.4 t ha^{-1}.

Table 2.12 Means for disease scores† and grain yield (t ha^{-1}) for the farmers' maize collections from Amazizi evaluated at Cedara and Baynesfield Estate in 2007/8 and 2008/9 seasons

PLS		GLS		NLB		Yield (t ha^{-1})	
Genotype	Mean§	Genotype	Mean	Genotype	Mean	Genotype	Mean
KL-2	7.7a	DL-1	7.7a	KL-1	5.7a	Kenyan	6.58a
KL-3	7.7a	NTL8-3	7.0ab	KL-3	4.7ab	KL-1	6.41ab
KL-1	7.5a	NTL8-4	6.8ab	KL-2	4.5b	DL-1	6.37ab
NTL8-1	6.3b	NTL8-2	6.8ab	Kenyan	4.0bc	NTL8-3	6.28ab
NTL8-4	6.0bc	NTL8-1	6.7ab	NTL8-4	3.3c	NTL8-4	6.28ab
NTL8-2	6.0bc	DL-2	6.7ab	DL-2	3.3c	KL-3	6.21ab
DL-1	5.7bc	DL-3	6.5ab	NTL8-1	3.3c	DL-2	6.02ab
DL-2	5.3cd	Kenyan	5.5bc	NTL8-3	3.3c	NTL8-2	5.80ab
NTL8-3	5.3cd	KL-1	4.5cd	DL-1	3.2c	NTL8-1	5.31abc
DL-3	4.7de	KL-2	3.5d	NTL8-2	3.2c	KL-2	4.80bc
Kenyan‡	4.3e	KL-3	3.2d	DL-3	3.2c	DL-3	3.85c
Mean	6.3		5.6		3.8		5.8
CV (%)	9.4		23.7		25.3		24.7
LSD(0.05)	0.7		1.6		1.1		1.7

†Disease rating scale used is indicated in section 2.2.6
‡Kenyan – was used as a check. It is a population that came from Kenya. §Means in each column followed by the same letter are not significantly different

Figure 2.2 Kenyan and Natal-8-row in the field showing disease symptoms

Symptoms of the diseases were often found on the same plant or different plants (Fig 2.2). Not all the plants in a row were susceptible to the same disease.

2.4.9 Farmers' perceived maize production constraints in Amazizi district

There were significant (P<0.001) differences in the farmers' responses for the different constraints (Table 2.13). Drought was rated as the number one constraint in Obonjaneni and Busingatha, whilst in Okhombe weeds were the number one constraint. A list of weeds that the farmers indicated were prevalent and problematic in the area is presented in Appendix 2.2. Generally, across the three villages, drought was the number one constraint, followed by excessive heavy rains, then storms and on fourth was soil fertility. Pest and diseases were ranked fifth and sixth. Unavailability of seed of other varieties or not enough seed were not important constraints to the farmers as they were only ranked number 17 and 18. Lack of resources to purchase inputs was also not important to the farmers and it was ranked number ten.

Table 2.13 Farmers' Perceived Maize Production constraints

Characteristic	Village			Mean	Rank‡
	Obonjaneni	Busingatha	Okhombe		
Drought	9.6 (92.0)†	8.7 (76.0)	5.8 (34.0)	8.1 (67.3)	1
Heavy rains	6.7 (45.0)	7.5 (57.0)	5.3 (28.0)	6.5 (43.3)	2
Storms	8.5 (73.0)	5.7 (33.0)	3.5 (12.0)	5.9 (39.3)	3
Soil fertility	7.0 (49.0)	4.5 (20.0)	5.8 (34.0)	5.8 (34.3)	4
Weeds	4.4 (19.0)	5.3 (28.0)	7.1 (50.0)	5.8 (32.3)	4
Insects	5.3 (28.0)	7.1 (51.0)	4.0 (16.0)	5.5 (31.7)	5
Diseases	5.0 (25.0)	3.7 (14.0)	3.2 (10.0)	4.0 (16.3)	6
Wrong planting time	2.0 (4.0)	4.8 (4.0)	3.7 (14.0)	3.5 (13.7)	7
Uncontrolled Livestock	2.0 (4.0)	2.0 (4.0)	4.0 (16.0)	2.8 (8.0)	8
Soil erosion	1.0 (1.0)	4.0 (16.0)	3.2 (10.0)	2.7 (9.0)	9
Not enough money for inputs	1.0 (1.0)	3.2 (10.0)	3.6 (13.0)	2.6 (8.0)	10
Poor land preparation	2.0 (4.0)	2.8 (8.0)	2.4 (6.0)	2.4 (6.0)	11
Wrong fertilizer type and (or) quantity	1.4 (2.0)	3.0 (9.0)	2.6 (7.0)	2.4 (6.0)	11
Scattered cattle	1.7 (3.0)	1.7 (3.0)	2.2 (5.0)	1.9 (3.7)	12
Water logging	1.0 (1.0)	1.4 (2.0)	2.2 (5.5)	1.6 (2.7)	13
Shortage of ploughing lands	1.7 (3.0)	1.4 (2.0)	1.4 (2.0)	1.5 (2.3)	14
Stony lands	1.0 (1.0)	1.7 (3.0)	1.7 (3.0)	1.5 (2.3)	14
Acidic soils	1.7 (3.0)	1.0 (1.0)	1.0 (1.0)	1.2 (1.7)	15
Lack of training in farming	2.0 (4.0)	0.0 (0.0)	1.4 (2.0)	1.1 (2.0)	16
Too much snow	2.4 (6.0)	0.0 (0.0)	0.0 (0.0)	0.8 (2.0)	17
Unavailability of other varieties of seed	1.4 (2.0)	0.0 (0.0)	1.0 (1.0)	0.8 (1.0)	17
Not enough seed	0.0 (0.0)	1.0 (1.0)	1.0 (0.0)	0.7 (0.7)	18
Baboons	1.0 (1.0)	0.0 (0.0)	0.0 (0.0)	0.3 (0.3)	19
Overall mean (transformed)				3.0	
P-value				<.001	
S.e.d				1.0	
Lsd (0.05)				2.0	

†Data transformed (square root transformation). Values in parenthesis are the untransformed percentages. Ranking based on transformed means. ‡The lower the rank, the more important the constraint

2.5 Discussion

2.5.1 General crop production aspects

Landholdings were relatively small, with an average of 1.4 ha per farmer. These landholdings were comparable in size to the observation made by Byerlee and Helsey (1997) that production of maize in Sub Saharan Africa (SSA) by smallholder farmers was on land holdings ranging from 0.5 to 3.0 ha. The PRA also established that maize was the principal crop in these three villages of Amazizi district as shown by the land allocated to its production. The farmers grew maize mainly for consumption but also for livestock feed. About 94 to 98% of the cultivated land was planted to maize. The few farmers who grew other crops planted vegetables, potatoes, beans and pumpkins. A few farmers had fruit trees such as peach and guava and they sold the fruits to supplement their incomes. Despite more land being allocated to maize, yields across the three villages were highly variable, ranging from a minimum of 0.2 to 5.7 t ha^{-1} depending on the variety grown. The low yields observed in these villages were comparable to yields reported by Pingali and Pandey (2001) for most smallholder farming sector in SSA which averaged below 1.2 t ha^{-1} against a potential of 7.0 t ha^{-1} The low yields were attributed to factors such as the majority of farmers being located in marginal areas with highly variable and stress-prone conditions thus indirectly forcing them to rely on low-input and low-risk cropping systems (Shumba, 1984; Banziger and de Meyer, 2002; Reeves and Cassaday, 2002). However, farmers in this district, although located in an above average agricultural potential area, relied mostly on low-input farming due to lack of capital, and this contributed to the low yields.

Results from the evaluation studies done by the principal investigator using the maize seed collected from the farmers in Amazizi district confirmed that the varieties grown by these farmers had high yield potential. Yields obtained ranged from 3.8 to 6.6 t ha^{-1}. Efforts should therefore be made to address the production constraints in the area that may be contributing to the low yields realized by farmers in these three villages.

2.5.2 Varieties of maize grown and how they were differentiated from each other

The selection of maize varieties grown by the farmers was not as diverse as has been reported for other communities. Almost all the farmers grew the local landrace NTL8 and a smaller percentage grew other varieties which included improved OPVs and Pannar hybrids (Table 2.2). The grain colour was mostly white for consumption and yellow for

livestock feed and a few other products that could be consumed. In total the farmers came up with about 12 varieties, with 77 to 100% of them growing NTL8 and only 1 to 39% growing the other varieties. Other communities, for example in Western Kenya, had about 20 varieties they grew, with about 8 local landraces to choose from (Odendo *et al*., 2002). Farmers in Manicaland area of Zimbabwe had more than 12 hybrids to choose from and one local landrace (Derera *et al*., 2006).

However, in this study, it appeared the adoption of hybrids was low, despite South Africa having many seed companies who produce hybrid seed. Only Pannar hybrids were being bought by the few farmers who grew them. The farmers had their own reasons for not growing hybrids, the main one being that seed was expensive and the hybrids needed extra inputs, which were also expensive. In contrast, the local landrace seed was recycled, and it was tolerant to acid soils and drought and they were assured of a harvest even during bad seasons unlike the hybrids. This finding is in agreement with reports by FAO and CIMMYT (1997) and Aquino *et al*. (2001) that although improved, superior cultivars have been developed in most of the countries in SSA, the majority of the small-scale farmers still rely on unimproved open-pollinated varieties (OPVs) for their plantings. This was partly because the OPVs were easy to multiply and therefore cheap and readily available (FAO and CIMMYT, 1997).

The most popular local landrace grown in Amazizi, NTL8 appeared to be closely related to the Hickory King (HK) variety based on the characteristics of the cobs. The HK was introduced in Southern Africa from the USA in 1905 (Weinamann, 1972). The variety is characterized by large dent kernels and can tolerate poor soils (McCann, 2005). There are now different versions of the HK available, ranging from six-rowed to ten-rowed, dent, semi-dent and semi-flint (Magorokosho, 2006). This landrace is still popular in Southern Africa as shown by collections done by Magorokosho (2006). Results from PRA by other researchers in Zimbabwe, Kenya and Zambia also indicated landraces with similar characteristics to the HK (Derera *et al*., 2006; Leley, 2007; Miti, 2007). In Zambia they called the landrace *Gangata* (Miti, 2007), whilst in eastern Kenya they called it *Kinyanya* (Leley, 2007) and in the eastern highlands of Zimbabwe they called it *Chitonga* (Derera *et al*., 2006). This suggests the local landraces being grown in eastern and southern Africa could all be related to the HK. The different variations of the local landraces could be a result of the hybridizations taking place in the field when the farmers grow other varieties. For example, in Amazizi district, the farmers indicated the DL variety was a hybrid

between the NTL8 and PAN hybrids and this variant has rows varying from 10 to 12, large grains and the seed is also recycled.

Farmers identified the varieties based mainly on the size of the kernels and number of rows per cob (Table 2.4). The local varieties; NTL8 and DL had large kernels and 8-12 rows per cob. In contrast the improved OPVs and PAN hybrids had small kernels and more than 12 rows per cob.

2.5.3 Farmers perceived advantages and disadvantages of the different maize types grown in their area

Farmers indicated that they preferred growing the local landrace mainly because it was tasty and less expensive as they could save seed, although it did not give high yields. It was also early in maturity and drought tolerant, giving satisfactory or enough yields (Table 2.5). This is in agreement with the findings by Magorokosho (2006) on landraces collected from Malawi, Zambia and Zimbabwe, whereby farmers kept landraces because of their taste, tolerance to most abiotic and biotic stresses, early maturity and yield stability. The few farmers who grew hybrids in Amazizi district preferred them mainly for the yield, disease resistance, white mealie-meal, and the fact that they were quick to dry and easy to shell. Most of these farmers grew the hybrids for sale and preferred them because they were also prolific, giving two to three cobs per plant. The improved OPVs were preferred mainly for recycling seed and yields which were higher than those obtained from the local variety and they were also resistant to the main biotic stresses.

2.5.4 Preferred maize traits by farmers from Amazizi district

Results of pair-wise ranking of traits showed the cost of seed as an important factor considered by farmers when choosing a variety (Table 2.7). Most farmers desired varieties with seed that could be recycled, as they did not have enough money to buy seed every season. Although, the farmers preferred growing their local variety for the taste, they still preferred high yield and they ranked it first. Taste was ranked second, although it was amongst the top perceived advantages of the local variety. Early maturity and low cost of inputs were also important characteristics considered by the farmers and were ranked first and second. Pests/diseases and drought were second to high yield. It appeared the farmers had developed their own mechanisms to escape diseases and drought. They indicated that they planted early to escape diseases and drought and this was also the reason why they preferred early maturing varieties.

According to the farmers, an “ideal” variety they preferred had to be high yielding, have good taste, inexpensive seed and should require minimum inputs. These were the top characteristics. The farmers indicated that they would want to grow hybrids for the high yield, but only if they could afford the seed and inputs required. Therefore opportunities do exist of improving the local landraces for yield and still maintain the other characteristics preferred by the farmers or introduce other improved varieties which incorporate the farmers’ preferences. When the farmers were given seed samples of various varieties to evaluate in the field, the farmers ranked Afric1 and KEP amongst the top varieties they preferred, despite only 1% and 26% of them having indicated growing Afric1 and KEP varieties, respectively, during the survesys and focus group discussions. These two are improved varieties and were preferred ahead of the hybrids and local varieties, mainly because there were tolerant to diseases and insects, resulted in white mealie-meal and yielded higher than the local varieties. In addition there were possibilities of recycling the seed unlike the hybrids.

The fact that the farmers selected these two improved varieties after the field evaluations, when only a small percentage of them were actually growing them reiterates the point of seed availability. The farmers indicated that they would want to grow hybrids and improved varieties for the high yield, but only if they could afford the seed and inputs required. In addition, the majority of the farmers cultivated maize purely for subsistence and there was therefore no incentive for them to buy maize seed when they anticipated no profit from it. However, opportunities do exist of improving the local landraces for yield and still maintain the other characteristics preferred by the farmers or introduce other improved open-pollinated varieties which incorporate the farmers’ preferences.

Diseases and pests were not ranked highly in most of the cases. In this study only 1% of the farmers indicated a disease with yellowish leaves through the structured survey. Odendo *et al*. (2002) in Western Kenya made a similar observation that diseases were always ranked low on the farmers’ perceived constraints list. It appears the symptoms of diseases are mostly confused with damage from abiotic stresses and pests. In this study almost all the farmers classified stalkborer damage as a disease not insect damage. Farmers were able to recognize diseases after being shown pictures which indicated the symptoms. Although they listed the diseases they had observed in their fields, the farmers indicated that the diseases did not occur frequently and did not cause any significant yield losses. Most diseases have been reported to be difficult to control because of their occurrence which is less predictable every season (Vivek *et al*., 2009).

Stalkborer and cutworms were the most important and prevalent pests and farmers used various methods to control these pests. However, despite the prevalence of these two pests, about 23 to 30% of the farmers did not use any chemicals to control them. This was mainly due to lack of resources.

2.5.5 Evaluation of farmers' maize collections for disease resistance and yield potential

Disease and yield evaluation of the maize varieties collected from the farmers demonstrated clearly that, although some of the varieties were susceptible, high levels of genetic variability existed within the different varieties. The KEP variety was susceptible to PLS, but resistant to GLS and moderately resistant to moderately susceptible to NLB. On the other hand NTL8 was susceptible to moderately susceptible to PLS, susceptible to GLS and resistant to NLB. The DL also gave varying reactions to the three diseases. The genetic variability that existed within the varieties could be exploited in breeding for disease resistance. The common rust pressure was low to allow any significant differentiation of the different varieties. There was a highly significant genotype x environment interaction. This was a result of different levels of disease in the two seasons and the two locations. Cedara had high PLS and GLS disease pressure in both seasons and high pressure for NLB in 2008/9 season, whereas Baynesfield Estate had high GLS pressure in 2008/9 season. The varieties also showed high potential for grain yield. Yields obtained in the evaluation trial for the different varieties ranged from 3.8 to 6.6 t ha^{-1} and were much higher than what the farmers obtained from their own plots. The variability in yield and disease reactions indicated that it was possible to select for high yield and disease resistant genotypes from these varieties. The results showed that the farmers' local varieties have high grain yield potential given the ideal conditions for growth. This implied that if the other production constraints were addressed and farmers produced maize with the recommended rates of inputs, they could realize high yields and thus reduce the gap that existed between their yields and potential yields.

2.5.6 Perceived maize production constraints

Farmers listed about 23 production constraints and drought was rated as the most important in Obonjaneni and Busingatha, whilst farmers in Okhombe rated weeds as the top constraint (Table 2.12). On the whole, drought was the top constraint across the three villages, followed by heavy rains, storms, soil fertility and weeds, insects, and diseases. According to the definition of good and bad seasons indicated by the farmers

(Appendix 2.4), drought meant poor distribution of rain during the season, especially poor rains during flowering and grain filling stages. Heavy excessive rains, hail storms and heavy winds were actually indicated by 65 to 77% of the farmers as a characteristic of a bad season (Appendix 2.4). The rainfall in the area is characterized by thunderstorms and intermittent dry spells. Not enough money for inputs was mentioned by only 8% of the farmers, although most of them indicated that they were not growing hybrids because the cost of seed and other inputs such as fertilizers was high. One would have, therefore, expected this constraint to be among the top constraints, but it was only ranked number ten. The reason could be that the farming in this area is more oriented towards subsistence and is based on low inputs. Very few farmers grow large acreages for sale and as a result there is no incentive in investing money into crop production.

On the other hand, soil fertility was listed as a major constraint. However, about 7 to 27% of the farmers did not use fertilizers and 5 to 58% did not apply manure (Appendix 2.2) to address the soil fertility problem. Those who used fertilizers did not use the recommended rates. The rate of compound fertilizer applied by the farmers ranged from 117 to 140kg ha^{-1} (Appendix 2.2). In addition, the farmers indicated in most cases that they used the wrong type and quantity of fertilizer due to ignorance and lack of technical advice and about 6% of the farmers cited this as one of the constraints.

2.6 Conclusions

The PRA established that maize was the principal crop grown in Amazizi district. The number of varieties grown was limited, with almost 100% of the farmers growing the local landrace which they called *Natal-8-row* or *IsiZulu*. Less than 40% of the farmers planted their fields to hybrids, or improved OPVs. The farmers preferred the local landrace mainly for its taste, seed which can be recycled, abiotic stress and yield stability. In terms of preferred characteristics of maize varieties, the top four were inexpensive seed, high yield, early maturity and low cost of inputs. Taste was not ranked that high although it was amongst the top perceived advantages of the local variety. Pests/diseases and drought were only ranked fifth and sixth. The farmers planted early to escape diseases and drought and this was also the reason why they preferred early maturing varieties. The local varieties had high yield potential and exhibited genetic variability for disease resistance that can be exploited in breeding programmes. Farmers indicated that they preferred varieties with high yield and were willing to grow hybrids if the cost of seed and other inputs were lowered. Abiotic stresses were amongst the top four constraints faced

by the farmers, whereas, biotic stresses were not ranked that highly. Breeding opportunities exist for breeding varieties resistant or tolerant to these abiotic stresses. There is great potential in raising yields of the local varieties by addressing the other production constraints which the farmers face. Work is currently in progress of selecting maize lines from the farmers' local varieties and there are promising lines (now in S_4) that have been developed which only need to be tested for combining ability for grain yield and disease resistance.

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2.8 Appendices

Appendix 2.1 Means for land holding (hectares), general crop production aspects goals of maize production and uses of maize in Amazizi district.

	Village			Overall mean	Min	Max	P-value[†]
	Obonjaneni	Busingatha	Okhombe				
Land holding and crops grown (hectares)							
Size of landholding	1.1	1.0	2.1	1.4	0.2	15.0	0.001
Size of cultivated land	0.9	0.8	1.9	1.2	0.1	14.8	0.001
Land for maize	0.8	0.8	1.8	1.1	0.1	14.8	0.001
Land for other crops	0.1	0.01	0.2	0.1	0.0	2.0	0.008
Time of planting (%)							
October	3.0	2.0	2.0				
November	64.0	69.0	73.0				
December	32.0	29.0	25.0				
Crops grown (% farmers growing)							Ranking
Maize	100.0	100.0	100.0	100.0			1
Pumpkins	2.0	9.0	9.0	6.7			5
Beans	3.0	3.0	8.0	4.7			4
Vegetables	7.0	4.0	1.0	4.0			2
Potatoes	7.0	3.0	4.0	4.7			3
Other[‡]	3.0	2.0	2.0	2.3			5
Goals of maize production (kg maize)							P-value
Home consumption	1824.0	354.6	625.0	924.6			0.001
Livestock feeds	119.9	78.8	143.5	114.1			0.003
Sale	1410.0	-	61.5	482.9			0.018
Purchase maize from others (%)							
Yes	33	21	45				
No	54	76	55				
Maize purchased from others (kg)							
Mean	17.3	18.4	114.4	52.3	0.0	400.0	0.000
Min	0.0	0.0	0.0				
Max	300.0	300.0	600.00				
Average yields (t ha^{-1})							
Mean	1.5	0.7	0.8				
Min	0.3	0.3	0.2				
Max	5.7	1.7	4.4				
Products made from maize							
Mealie meal	89.0	98.0	96.0	94.3			
Samp	68.0	82.0	72.0	74.0			
Green mealies (boiled or roasted)	87.0	58.0	67.0	70.7			
Mealie bread	49.0	41.0	40.0	43.3			
Porridge	80.0	90.0	82.0	84.0			
Livestock feed (yellow maize)	55.0	46.0	36.0	45.7			
Mealie rice	16.0	20.0	60.0	32.0			
[§]Other maize products	31.0	45.0	51.0	42.3			

[†]Probability values based on Kruskal-Wallis ANOVA. [‡]Other crops included mainly sorghum and fruits. §Other products were mostly Zulu traditional dishes given with Zulu names.

Appendix 2.2 : Soil management in Amazizi district

	Village			Overall mean	Min	Max	P-value
	Obonjaneni	Busingatha	Okhombe				
Fertilizer usage and type							
none	27.0	20.0	7.0				
3:2:1	51.0	67.0	68.0				
†Other	20.0	13.0	25.0				
unknown	2.0						
Fertilizer amount (kg)	114.0	117.5	223.1	153.4	0.0	5750.0	0.079
Fertilizer rate (kg/ha)	131.0	140.0	117.3	127.2			
Manure usage and sources							
None	58.0	15.0	5.0				
cattle	26.0	54.0	57.0				
goats	2.0	5.0	27.0				
poultry	13.0	26.0	17.0				
‡other		3.0	4.0				
Manure amount (kg)	37.6	104.2	170.0	106.5	0.0	1600.0	0.000
Manure rate (kg ha^{-1})	43.7	124.1	89.4	88.1			

†Other fertilizer = superphosphate, DAP, 3:2:4, unknown
‡Other sources of manure = pigs, horses, sheep

Appendix 2.3 List of problem diseases, pests and weeds (% farmers responding)

	Village		
	Obonjaneni	Busingatha	Okhombe
Diseases and pests			
yellowish leaves	1		
stalkborer	71	74	78
cutworms	80	67	68
†Other	58	42	79
Problem diseases and pests			
none	30	29	23
stalkborer	69	71	77
cutworms	69	53	42
weevils	6	2	
Other	10	18	25
Weeds			
mnyankomo	61	81	86
curcuva grass	34	72	77
blackjack	68	52	56
datura	14	15	14
‡Other	42	40	41
Difficult to control weeds			
mnyankomo	38	39	40
curcuva grass	31	41	60
blackjack	18	1	2
Other	18	34	16

†Other insects = red and black insects, white butterflies, rats, moles, ants, “mkhothane”
‡Other weeds = most were indicated with Zulu names.

Appendix 2.4 Chemicals used by the farmers to control pests and diseases in their area

	Village		
	Obonjaneni	Busingatha	Okhombe
Control of pests and diseases			
no control	75	87	78
unknown pink granules	3	2	6
unknown white chemical	4	2	1
unknown blue chemical	5	1	2
stalkborer granules	5	10	12
Kemprin 200EC	3	-	-
Phostoxin tablets	3	-	1
Jeyes fluid and pill	3	1	1
Other (LTA	-	-	1
Jeyes fluid	7	-	-
Jeyes fluid + sea water	1	-	-
blue death mixed with fertilizer	2	-	4
malasol	2		-
salt added to manure(or fertilizer) and seed	9	1	9
paraffin + salt added to seed	1	1	1
pepper	1	-	-
salt and pepper	1	-	-
sunlight liquid	1	-	-
green powder mixed with fertilizer	-	1	-
Weed Control			
hoeing	95	99	99
herbicides	5	-	1
Herbicides Used			
none	85	89	84
unknown	5	5	2
roundup	5	1	-
seedflo	1	-	-
gramoxone before planting	2	-	-
tollazine after planting	1	-	-
white chemical	3	1	6
blue chemical	1	1	-

Appendix 2.5 Definition of good and bad seasons according to the farmers in Amazizi (% of farmers suggesting the characteristic)

Good Season	%	Bad Season	%
Timely rains – especially in October, for timely planting of crops	4.0	Late rains – delays planting	4.0
Good distribution of rain throughout the season	99.3	Heavy excessive or continuous rains	77.3
Enough sunlight	50.3	Too many overcast days	50.3
Moderate weather – not too hot, cold or windy	2.3	Drought, especially during flowering and grain filling stages	98.3
		Hail storms and heavy winds	65.6
		Snow before harvesting	2.7
		Too many insects	1.3

3 Genetic variability of Tropical Maize Germplasm for Phaeosphaeria Leaf Spot Disease Resistance under Field Conditions

Abstract

Phaeosphaeria leaf spot (PLS), caused by *Phaeosphaeria maydis* (Henn.), is becoming a major disease in maize production in sub-Saharan Africa (SSA). However, sources of resistance to PLS are still limited, especially in germplasm adapted to tropical and subtropical environments of Africa. This study was therefore conducted to evaluate maize germplasm adapted to tropical and subtropical environments of Africa for PLS resistance and to monitor progress of the disease in the field. Seventy-six inbreds and populations, and 64 experimental and commercial hybrids were evaluated from 2006/7 to 2008/9 seasons at Cedara Agricultural Research Station in South Africa. Disease resistance evaluation was based on standardized area under disease progress curves (SAUDPC) and final PLS disease severity scores. Disease development was variable from season to season, resulting in different levels of PLS severity, thus contributing to a highly significant ($P \leq 0.001$) genotype x environment interaction observed for both inbreds and hybrids. Significant ($P \leq 0.05$) variation was observed among the inbreds, populations and hybrids. Overall, 63% of the inbreds/populations were symptomless, resistant or moderately resistant to PLS. Some of the regionally important inbred lines like SC and N3, and CIMMYT's most successful lines such as CML395, CML444, CML202, CML312, and CML488 were resistant. Fifty-four percent of the single-cross experimental hybrids and 46% of the commercial hybrids were resistant to PLS. Correlation coefficients between SAUDPC values for disease severity with PLS scores were significant ($P<0.001$) and positive. This implied that ranking of the genotypes for SAUDPC and final PLS disease severity score was generally similar. The SAUDPC for disease severity were significant ($P<0.001$) and negatively correlated with flowering days (50% to anthesis and silking). PLS disease was observed after flowering, but for most of the susceptible genotypes, the disease progression during the season was rapid. Resistance was moderate to highly heritable implying that phenotypic selection would be effective in breeding for PLS resistance. The results clearly demonstrated that high levels of resistance were available in the regionally adapted germplasm and additional sources of resistance were identified. The experimental hybrids that exhibited high levels of resistance can be recommended for further testing and release.

3.1 Introduction

Diseases are amongst the major constraints contributing to low yields to maize production worldwide (Vivek *et al*., 2001). Although not reported in epidemic proportions, Phaeosphaeria leaf spot (PLS) disease is becoming increasingly important in the region. It has been reported from Kenya (Njuguna *et al*., 1992), South Africa (Smit and Lawrance, 2004) and Zimbabwe (Levy, 1996; Derera *et al*., 2007), Cameron (Carson, 1999) and other reports have been from Uganda, Rwanda and Zambia. In Brazil, where PLS is a major disease, severe yield losses of more than 60% in susceptible maize cultivars have been reported (Casela, 1998). The increase in PLS disease was attributed to practices such as late planting, absence of rotation, and zero tillage practices (Casela, 1998; Cervelatti *et al*., 2002). In general, these practices promote the build-up of sufficient inocula over the seasons.

Diverse sources of PLS resistance have been identified and reported by a number of researchers (Das *et al*., 1989; Pegoraro *et al*., 2002, Silva and Moro, 2004; Mhembere, 2005; Derera *et al*., 2007, Vivek *et al*., 2009). However, majority of these studies have been conducted on temperate materials and on yellow maize and therefore cannot be used directly by most African farmers in their tropical environments unless they undergo extensive local adaptation. There are a few sources of resistance that have been identified in African germplasm (Derera *et al*., 2007; Vivek et al., 2009). However, information is still limited on PLS resistance which is available in the important tropical inbreds that dominate hybrid parentage in tropical Africa. Therefore given the importance of maize and the diverse maize production environments in Africa, more sources of resistance would be essential. Focus should, therefore, be on finding sources of PLS resistance from the major heterotic groups that are adapted to subtropical and tropical environments and are widely used in breeding programmes in Africa. The purpose of this study was therefore; i) to screen maize germplasm that are adapted to tropical and subtropical environments of Africa to identify sources of resistance to PLS, ii) to monitor the progress of PLS disease in the field, and iii) to estimate heritability of PLS resistance.

3.2 Materials and methods

3.2.1 Germplasm sources

Germplasm screened included maize inbred lines obtained from the CIMMYT programme in Harare, Zimbabwe, the Crop Breeding Institute in Zimbabwe, collections from farmers

in Amazizi district, KwaZulu Natal in South Africa and Kenya. The inbred lines were sampled from the major heterotic groups that are adapted to subtropical environments and are indicated in Table 3.1 and Table 3.2. The important heterotic groups included the broad CIMMYT A and B classification (CIMMYT, 2001), the SC (Southern Cross), N3 (derived from Salisbury white), K64r derivatives and the "P" heterotic group (derivatives from Natal Potchefstroom Pearl) (Gevers and Whythe, 1987; Olver, 1998; Derera, 2005) amongst others. The CIMMYT group A is mainly derived from populations like the Tuxpeno, Kitale, BSSS (Iowa Stiff Stalk Synthetic), B73 and N3, whereas CIMMYT group B are derivatives from populations such as the ETO, Ecuador 573, Lancaster, Mo17 and SC (CIMMYT, 2001).

A total of 76 inbred lines, populations and collections from farmers were screened for PLS resistance. These included advanced elite lines, amongst them the most successful and promising lines from CIMMYT (CIMMYT, 2001). In addition, a total of 64 experimental hybrids developed at the African Centre for Crop Improvement (ACCI), University of KwaZulu-Natal (UKZN) in South Africa and 12 commercial hybrids, supplied courtesy of Seed Company (Seedco, Zimbabwe) and Pannar hybrid seed, (South Africa). The other hybrid AFRIC 1 (AF1) was bought from the retail outlet, AFGRI, Pietermaritzburg in South Africa. These commercial hybrids were evaluated alongside the experimental hybrids in order to determine their reaction to PLS.

3.3 Experimental site and design

The study was carried out at Cedara Agricultural Research Station in South Africa (30°16'E, 29°32'S, 1130 metres above sea level (m.a.s.l) from 2006/7 to 2008/9 seasons. The inbred lines and hybrids were evaluated in separate experiments over three seasons. In 2006/7 season 45 inbreds and populations were screened, and the number was increased to 72 in 2007/8 and 2008/9 seasons. The hybrids were screened in 2007/8 season (two planting dates) and 2008/9 season. Inbreds and populations were planted in January 2007 for the 2006/7 season (C07), November 2007 for the 2007/8 season (C08), and November 2008 for the 2008/9 season (C09). Hybrids were planted in November 2007 for C108, January 2008 for C208 and November 2008 for C09. The row-column alpha designs were used in all the seasons. In 2006/7 a 15 x 3 row-column design was used, with 2 replications for the inbred lines and populations. In 2007/8 and 2008/7 seasons, the design was a 9 x 8 alpha (0,1) lattice for the inbreds and a 9 x 6 alpha (0, 1) lattice design for the hybrids. Some of the hybrids and inbreds screened in the first season were not included in the second and third seasons due to seed shortages.

Table 3.1 Inbred lines evaluated for disease resistance between 2006/7 and 2008/9 seasons at Cedara Agricultural Research Station

Line/Population	Pedigree	Heterotic grouping
A1220-4	[(CML395/CML444)-B-4-1-3-1-B/CML395//SC/ZM605#b-19-2-X]-1-2-X-1-1-BBBBBB]-7-1-3-2-BBB	B / SC
A13	[[EV7992]C1F2-430-3-3-3-X-7-B-B/CML202]-6-2-2-3-B-B	A
A15	[CML197/N3//CML206]-X-32-1-4-B-B-B-B	N
B17	[LZ956441/LZ966205]-B-3-4-4-B-5-B-B-B-B	B
B18	Z97SYNGLS(B)-F2-188-2-1-3-B	B
CIM58	[P502C2/INTB-91-1-2-2-B]-2-6-1-1-2-2-B	B
CIM59	[P502C2/INTB-91-1-2-2-B]-2-6-1-1-3-1-B	B
CM31	TS3 LPA1-1	-
CM33		-
CM34	C063 LPA1-1	-
CM35		-
CML202	ZSR923S4BULK-5-1-b-b	B
CML205 (MP82)	[EMSR]#B#bF101sr-2-1-sr-3-2-4-b-b	B
CML312	S89500F2-2-2-1-1-B*5	A
CML312P	derived from CML312 (S89500F2-2-2-1-1-B*5)	A
CML373	P43SR-4-1-1-2-1-B-8-1-BBB	A
CML395(P3)	90323(B)-1-B-1-B*4	B
CML440	G16SeqC1F47-2-1-2-1-BBBBBB	A
CML441	ZM605C2F1-17-1-B-1-BBB	B
CML442B	[M37W/ZM607#bf37sr-2-3sr-6-2-X]-8-2-X-1-BBBBBBB	A
CML443	[AC8342/IKENNE{1}18149SR//PL9A]C1F1-500-4-X-1-1-BB-1-BBBBB	AB
CML444(CZL99029)	P43C9-1-1-1-1-1-BBBBBBB	B
CML445	[[TUXPSEQ]C1F2/P49-SR]F2-45-7-5-1-BBB	AB
CML488	DTPWC8F31-4-2-1-5-BBB	B
CML489	(CML202/LPSC3H297-2-1-1-2-2-#)-B-3-1-1-8-BB'	B
CML504(CZL99014)	[COMPE2/P43-SR//COMPE2]FS#20-1-1-B-1-BBB	A
CZL00001	INTA-191-2-1-2-BBBBB	A
CZL00003	DRB-F2-60-1-1-1-BBBB	B
CZL00009	INTA-F2192-2-1-1-1-BBBBB	A
CZL00018	DTPWC8F31-4-2-1-6-B	B
CZL00029(P20)	SNSYNF2[N3/TUX-A-90]-57-X-3-4-B-3-B-B	A / N3
CZL00032	[[NAW5867/P30SR]-40-1/[NAW5867/P30SR]-25-1]-17-2-2-B-1-B*2	A / P
CZL00034	CZL00034	-
CZL01002	[P501c2/[EV7992#/EV8449-SR]C1F2-334-1(OSU8i)-1-1-X-X-BB]-4-1-1-4-1-B*4	A
CZL03003	[[[K64R/G16SR]-39-1/[K64R/G16SR]-20-2]-5-1-2-B*4/CML390]-B-38-1-B-7-#-B	B / K
CZL99013(MP70)	[NAW5867/P49SR(S2#)//NAW5867]F#-48-2-1-B-2-B-7-BB-1-B-#-B*4	A / P
E6	QPM	
Early-34	ZM303c1-32-3-B-1-1-B-B	-
MP1	ZEWAc1F2-254-2-1-B-1-B-B	-
MP12	NIP25-100-1-1-B-1-B-B	-
MP13	NIP25-230-2-1-B-1-B-B	-
MP18	[[[NAW5867/P30SR]-111-2/[NAW5867/P30SR]-25-1]-9-2-3-B-2-B/CML388]-B- 35-2-B-1-#-1-BB	A / P
MP5	ZEWAc1F2-84-2-1-B-1-B	-
MP58	[MSRXG9]C1F2-205-1(OSU23i)-5-3-X-X-1-BBB-1-BB	-
MP59	Ac8342/IKENNE{1}8149SR//PL9A]C1F1-500-4-X-1-1-BB-1-BBB	AB
MP66 (CML485)	89[G32/DRSTEW]#31-1-2-BB-3-5-2-B-1-2-6-BBB	A
MP77	[SW1SR/COMPE1-W###S2#]-19-5-1-BBB-4-BB-1-2-BB-B	-
MP78	[P501c2/[EV7992#/EV8449-SR]C1F2-334-1(OSU8i)-1-1-X-X-B-B]-4-1-1-4-1-1-6-B	A
N2109	(Lancaster Oh43][CML352)S1-B	AB
N3	Salisbury White	N3
NAW5885	Potchefstroom Pearl	A / P
P12 (CZL00008)	[SW1SR/COMPE1-W###S2#]-126-2-1-B*3	A
P5 (CZL99027)	ZM605C2F1-17-1-B-1-BBB	B
P6 (CZL99028)	G16SeqC1F47-2-1-2-1-BBBBBB	A
PN7(T2)	PN7-2B (temperate B derived line)	B
PN8(T3)	PN8-B (temperate B derived line)	B
SC	Southern Cross	B / SC
T4	Temperate AB derived line	AB

Heterotic group A: derivatives from N3, NAW, Tuxpeno, Kitale, B73, BSSS (Iowa Stiff Stalk Synthetic)
Heterotic group B: derivatives from SC, K64R, Eto, Ecuador, Mo17, Lancaster

Table 3.2 Populations evaluated for disease resistance between 2006/7 and 2008/9 seasons at Cedara Agricultural Research Station

Line/Population	Pedigree
DL-1	Doylanda
DL-2	Doylanda
DL-3	Doylanda
Kenyan	Kenyan
KL-1	Kalahari Early Pearl
KL-2	Kalahari Early Pearl
KL-3	Kalahari Early Pearl
NTL8-1	Natal-8-row
NTL8-2	Natal-8-row
NTL8-3	Natal-8-row
NTL8-4	Natal-8-row
QM3	QPM
QM5	QPM
QM6	QPM
QM7	QPM

The plot size for both the hybrids and inbred lines/populations in each season was two rows, 3 m long, with 0.75 m inter-row spacing and 0.3 m intra-row spacing. Plant population densities were about 44 000 per hectare in all the seasons. A susceptible maize hybrid and inbred line were used as borders for the hybrid and inbred trials, respectively. Fertiliser was applied at the rate of 120 kg N, 33 kg P, and 44 kg K. Standard cultural practices including ploughing and disking, hand planting, hand weeding and/or application of herbicides and fertilizers were followed at each site.

Phaeosphaeria leaf spot (PLS) disease severity was assessed on a ten-day or fortnightly interval from the first appearance of symptoms, based on visual assessment of the whole plot. A 1-9 logarithmic rating scale was used where 1 = 0%, 2 = <1%, 3 = 1-3%, 4 = 4-6%, 5 = 7-12%, 6 = 13-25%, 7 = 26-50%, 8 = 51-75% and 9 = 75-100% leaf area showing disease symptoms. The scores were further classified into the following disease reaction types; 1.0 = symptomless, 2.0-4.0 = resistant, 4.1-5.0 = moderately resistant, 5.1-6.0 = moderately susceptible, 6.1-9.0 = susceptible. Disease incidence was assessed in 2008/9 season by counting the number of plants that showed PLS symptoms in each plot. The numbers were then expressed as percentages of the total number of plants per plot. Flowering dates, that is, days to 50% anthesis and silking were recorded for all the genotypes following the standard practice used at CIMMYT (CIMMYT, 1985).

3.4 Data analysis

The percentage disease incidence values were transformed using square root (x + 1). Area under disease progress curves (AUDPC) values were calculated for the disease severity scores and transformed incidence data. The following formula (Wilcoxson *et al*., 1975) was used for AUDPC:

$$AUDPC = \sum_{i=1}^{k} \tfrac{1}{2}(S_i + S_{i+1})(t_{i+1} - t_i)$$

where: S_i = score of severity or incidence at days *i*, and *k* is the number of scores, t_i is the time at *i* days.

Area under disease progress curves were standardized by dividing the AUDPC values by the total time duration of the epidemic in each season (Fry, 1977) to allow for comparisons between the two seasons. The duration of the epidemic was 59 days in 2007/8 season and 76 days in 2008/9 season.

The data from the last PLS scores was used for the analysis. Data from individual seasons and from combined seasons were analysed using PROC GLM procedure in SAS 9.1 (SAS Institute, 2002). The data were subjected to ANOVA firstly by environment with genotypes as the main effect, then a combined analysis across environments was conducted to analyse the effect of years, genotypes and interactions. A mixed model was used where genotypes were fixed and environments random. Genotype means were compared using the t-test (P=0.05). Pearson correlation coefficient values were calculated for selected parameters using the SAS procedure, PROC CORR (SAS Institute, 2002).

3.4.1 Heritability estimates for PLS disease

The reference population used for heritability estimates was the inbred lines. Variance components were calculated by equating the mean squares from the analysis of variance (ANOVA) estimates to the expectations of mean squares (EMS) (Table 3.3). Estimations were done on the combined environments. Single environments usually give high heritability than the combined environments because of low environmental variance and also the number of replications was low.

For the combined environments the following model was used (Singh *et al.,* 1993):

$Yij = \mu + gi + \alpha k + \delta ik + \beta jk + \varepsilon ijk$

Where: *where: Yijk = the response of ith genotype grown in the jth block (i = 1, 2...v), jth block (j = 1... b) over the kth environment (k = 1... L). μ = general mean, gi = the effect of the ith genotype, αk = effect of the kth environment, δik = the interaction effect between the ith*

genotype and the kth environment and βjk = effect of the jth block within the kth environment. The effects gi, δik and ε are assumed to be independently and normally distributed with zero mean and variances σ^2_g, σ^2_{gxe} and σ^2_e).

Table 3.3 ANOVA and the expected mean square (EMS) used for estimating variance components

Source	Df	Mean Square	EMS
Environment	2		
Rep(Env)	3		
Genotype	58	M1	$\sigma^2_e + r\sigma^2_{g*e} + er\sigma^2_g$
Genotype*Env	83	M2	$\sigma_e + r\sigma^2_{g*e}$
Error	164	M3	σ_e

The broad-sense heritability was then estimated as $\sigma^2_g / (\sigma^2_g + \sigma^2_{gxe} + \sigma^2_e)$, where σ^2_g = genetic variance, σ^2_e = environmental variance and σ^2_{gxe} = variance of the genotype x environment interaction.

3.5 Results

Analysis of variance for the final PLS disease severity scores are presented in Table 3.4. Mean squares for genotypes and the genotype x environment interactions were highly significant (P≤0.001) for PLS diseases in both the inbreds, populations and the hybrids. The environment was significant (P<0.001) for the populations and hybrids and not for the inbreds

Table 3.4 Combined Analysis of Variance for PLS disease severity scores for the inbreds, populations and hybrids screened between 2006/7and 2008/9 seasons

	Inbreds		Populations		Hybrids	
Source	DF	Mean Square	DF	Mean Square	DF	Mean Square
Environment (Env)	2	2.94^{ns}	2	22.04***	2	31.06***
Rep(Env)	3	1.55^{ns}	3	2.21*	3	4.45**
Genotype	58	23.45***	14	9.06***	64	18.19***
Genotype*Env	83	3.45***	13	6.19***	95	2.54***
Error	164	1.89	26		159	0.96
Corrected Total	310		58		323	
CV (%)	29.38		12.09		19.54	

*, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001, respectively, ns indicates non-significant (P>0.05).

3.5.1 Reaction of inbreds and populations to PLS disease infection

The severity scores for the inbreds and populations were variable and the differences were significant (P=0.05) in the different seasons (Table 3.5). The severity scores ranged from 1.0 to 9.0 across the seasons. The mean PLS was higher in 2008/9 season (C09) than in the other two seasons. In 2006/7 (C07) and 2007/8 (C08) seasons, PLS mean scores were 1.0 and 0.8 respectively, lower than in C09. Disease pressure was high in all the three seasons as shown by the maximum scores for the most susceptible inbred lines and populations. About 60-80% of the inbred lines and populations in the different environments were consistently symptomless to moderately resistant (scores 1.0 to 5.0) based on the classification used in this study. Overall, across environments, about 58% of the inbreds and populations were resistant or moderately resistant and only 5% were symptomless, that is either immune or escapes (Fig 3.1). Some of the regionally important inbred lines like SC and N3 and CIMMYT's most successful lines such as CML395, CML444, CML202, CML312, and CML488 were resistant to moderately resistant with scores ranging from 2.0 to 4.5 (Table 3.5). In contrast, the collections from the farmers, that is, the NTL8s, DLs and KLs were all susceptible with scores ranging from 6.5 to 9.0 in the two seasons they were tested. The inbred line MP70 had the highest score of 9.0 in all the three seasons.

Most of the inbreds and populations were consistent in their disease reactions in the different environments. However, there were a few genotypes that had variable reactions in the three environments. For example, CZL00009 had a score of 4.0 in C07, a score of 7.0 in C08 and 4.8 in C09 and Early34 had scores ranging from 5.0 to 8.0 in the different environments.

Table 3.5 Means for PLS scores in inbreds and populations screened between 2006/7and 2008/9 seasons

†C07		C08		C09		Combined		Disease Reaction‡
GENOTYPE	Mean	GENOTYPE	Mean	GENOTYPE	Mean	GENOTYPE	Mean	
MP70	9.0	MP70	9.0	MP70	9.0	MP70	9.0	S
CM35	9.0	CM35	9.0	NTL8-4	9.0	CM35	9.0	S
MP58	7.5	CM34	9.0	CM35	9.0	CM34	8.8	S
CML205	7.5	P12	8.5	KL-1	9.0	NTL8-1	8.5	S
CML443	7.0	NTL8-1	8.5	CM34	8.5	KL-1	8.5	S
CZL00032	7.0	NTL8-2	8.0	QM7	8.5	NTL8-4	8.3	S
MP13	6.5	MP58	8.0	NTL8-2	8.5	NTL8-2	8.3	S
		KL-1	8.0	NTL8-1	8.5	KL-2	8.0	S
		KL-3	8.0	KL-2	8.5	P12	8.0	S
		Early34	7.5	Early34	8.0	KL-3	8.0	S
		NTL8-4	7.5	KL-3	8.0	MP58	7.8	S
		KL-2	7.5	DL-2	8.0	DL-1	7.5	S
		DL-1	7.0	CIM58	8.0	NTL8-3	7.5	S
		NTL8-3	7.0	QM6	8.0	DL-2	7.5	S
		MP66	7.0	DL-1	8.0	Early34	7.1	S
		CIM58	7.0	NTL8-3	8.0	P5	7.0	S
		CIM59	7.0	Kenyan	7.5	CIM58	7.0	S
		CZL00009	7.0	DL-3	7.5	CZL00032	7.0	S
		P5	7.0	P12	7.5	DL-3	6.5	S
		DL-2	7.0	CIM59	7.0			S
		CML441	7.0	CML441	6.5			S
		T4	6.5					S
CML441	6.0	NAW5885	6.0	CML205	5.8	T4	6.0	MS
CIM58	6.0	DL-3	5.5	CZL00001	5.7	CML205	6.0	MS
E6	6.0			T4	5.5	QM6	5.8	MS
						Kenyan	5.8	MS
						E6	5.5	MS
						CML441	5.5	MS
						MP13	5.3	MS
						CZL00001	5.3	MS
						CZL00009	5.1	MS
						CML443	5.1	MS
CIM59	5.0	E6	5.0	CZL00009	4.8	MP59	4.8	MR
NAW5885	5.0	MP59	5.0	CML443	4.8	CM34	4.8	MR
Early34	5.0	CML312	4.5	CML504	4.5	NAW5885	4.7	MR
CZL00001	5.0	CZL00001	4.5	N2109	4.5	MP66	4.3	MR
A13	4.5			QM3	4.5	QM7	4.3	MR
MP59	4.5							
CML504	4.0	CML205	4.0	A13	4.0	A13	3.8	R
MP18	4.0	QM6	4.0	CML442-B	4.0	QM3	3.8	R
CZL03003	4.0	Kenyan	4.0	A1220-4	3.8	CZL03003	3.8	R
CZL00009	4.0	CML443	4.0	CZL00003	3.5	CML312	3.6	R
CML202	3.5	CML373	4.0	A15	3.5	CML504	3.6	R
QM7	3.5	CML445	4.0	CML312	3.5	CML373	3.5	R
QM5	3.5	A1220-4	3.5	MP66	3.0	P6	3.5	R
CML445	3.5	PN7(T2)	3.5	P20	3.0	CML445	3.4	R
CML312	3.0	CZL03003	3.5	CML489	3.0	PN7(T2)	3.3	R
CML504	3.0	P6	3.5	PN7(T2)	3.0	A1220-4	3.0	R
N2109	2.5	QM3	3.0	PN8	3.0	CZL99014	3.0	R
A15	2.5	A13	3.0	CML312P	3.0	A15	2.8	R
SC	2.5	CML504	2.5	CML373	3.0	CML312P	2.8	R
CML488	2.0	A15	2.5	MP18	3.0	MP18	2.8	R
B18	2.0	CML312P	2.5	CML202	3.0	N2109	2.7	R
MP5	2.0	MP13	2.5	NAW5885	3.0	CML202	2.7	R
P20	2.0	B18	2.0	MP77	3.0	SC	2.5	R
CML440	2.0	CML395	2.0	CML445	2.7	CML442-B	2.5	R
MP1	2.0	CZL00003	2.0	MP12	2.5	QM5	2.5	R
MP77	1.5	CML202	2.0	CML440	2.5	P20	2.2	R
CML444	1.0	CZL01002	2.0	N3	2.5	CZL00003	2.2	R
MP12	1.0	CML489	2.0	B17	2.0	CML489	2.0	R
A1220-4	1.0	P20	1.5	CM31	2.0	B18	2.0	R
CZL00018	1.0	N3	1.5	CML444	2.0	CML440	2.0	R
CZL01002	1.0	CML440	1.5	CML488	2.0	N3	1.9	R
B17	1.0	PN8	1.5	MP78	1.5	CML488	1.9	R
CML395	1.0	CML488	1.5	CZL00018	1.5	PN8	1.8	R
CML489	1.0	B17	1.5	CML395	1.5	CZL01002	1.5	R
CZL00003	1.0	MP78	1.5	CM33	1.0	CML395	1.5	R
N3	1.0	QM5	1.5	MP5	1.0	MP1	1.5	R
		MP18	1.0	PN8(T3)	1.0	CM31	1.5	R
		CML444	1.0			B17	1.5	R
		MP5	1.0			MP78	1.5	R
		CZL00018	1.0			CML444	1.3	R
		CML442-B	1.0			MP5	1.3	R
		CZL00034	1.0			CZL00018	1.3	R
		CM33	1.0			MP77	1.3	R
		MP1	1.0			MP12	1.2	R
		MP12	1.0			CZL00034	1.0	R
		CM31	1.0			CM33	1.0	R
		N2109	1.0					
		QM7	1.0					
		MP77	1.0					
Mean	3.6		3.8		4.6		4.2	
CV (%)	34.4		31.5		26.2		28.6	
LSD (0.05)	2.6		2.4		2.4		1.7	

†C07 = Cedara January 2007 planting (2006/7 season), C08 = Cedara, November 2007 planting (2007/8 season), C09 = Cedara, November 2008 planting (2008/9 season). ‡Disease reactions: R = resistant, MR = moderately resistant, S = susceptible and MS = moderately susceptible.

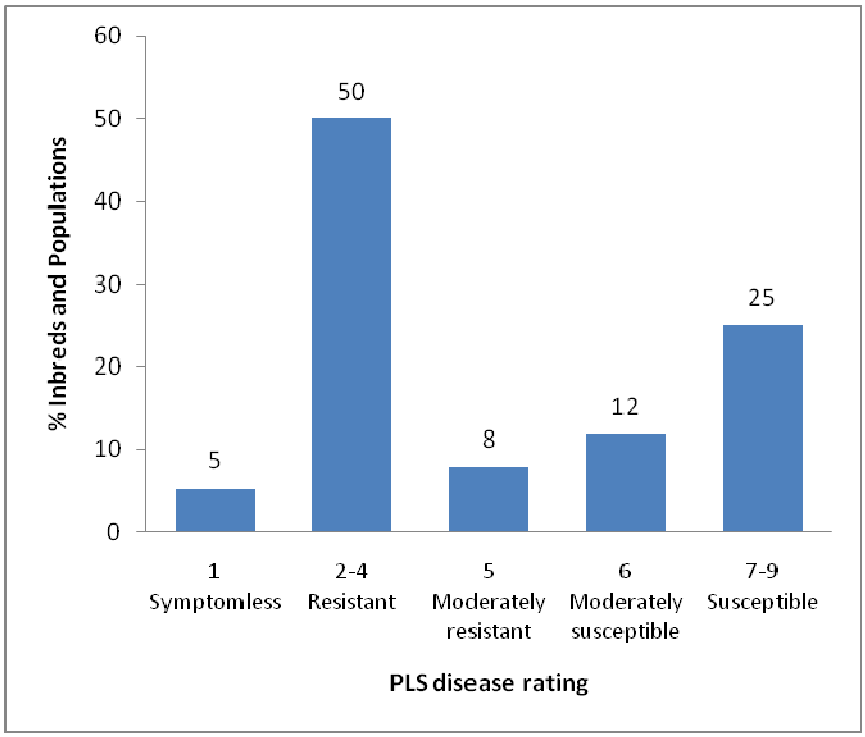

Figure 3.1 Frequency distribution of mean Phaeosphaeria leaf spot ratings of inbreds and populations evaluated over three seasons at Cedara Agricultural Research Station

3.5.2 Reaction of hybrids to PLS disease infection

There were significant differences (P=0.05) amongst the commercial and experimental hybrids for PLS disease in the different environments (Table 3.6). The hybrid reactions followed almost a similar trend as the inbreds/populations. The scores in the different environments ranged from 1.0 to 9.0. The commercial hybrid checks were amongst the most susceptible to PLS in all the three environments. In general the maximum scores observed for the most susceptible hybrids ranged from 8.0 to 9.0 in the three environments. The mean PLS was higher in C208 than in C108 and C09. In C07 and C08, PLS mean scores were 1.3 and 0.7 respectively, lower than in C09 (Table 3.6). No hybrids were symptomless in C208. Of the commercial hybrid checks tested, more than 60% overall, had scores from 5.3 to 8.5 (moderately susceptible to susceptible). Fifty-four percent of the experimental hybrids and 36 % of the commercial hybrids had scores ranging from 1.5 to 5.0 (resistant to moderately resistant) (Fig 3.2). There were also a number of hybrids that had variable reactions in the three environments. A few examples of these include EH30 which had scores of 3.0, 5.5 and 7.5, EH38 scores ranged from 4.5 to 7.0 and EH1 had scores from 3.0 to 6.0 in the different seasons.

Table 3.6 Mean scores for experimental and commercial hybrids screened between 2006/7and 2008/9 seasons

C108		C208		C09		Combined		
Variety	Mean	Variety	Mean	Variety	Mean	Variety	Mean	Disease Reaction
P17	8.5	S40	8.0	P17	9.0	P17	8.5	S
P77	8.0	S51	8.0	P77	8.5	P77	8.2	S
S40	8.0	P17	8.0	EH43	8.0	P14	8.0	S
EH8	8.0	EH2	8.0	S51	8.0	EH43	8.0	S
EH2	8.0	EH28	8.0	EH20	8.0	S51	8.0	S
EH6	8.0	EH5	8.0	EH30	7.5	EH20	8.0	S
P27	8.0	EH3	8.0	P067	7.5	S40	8.0	S
EH5	7.5	EH7	8.0	EH7	7.5	EH2	7.5	S
EH3	7.0	EH8	8.0	P27	7.0	EH5	7.5	S
P57	7.0	EH36	8.0	EH38	7.0	EH8	7.3	S
P067	6.5	EH22	8.0	EH5	7.0	EH36	7.3	S
EH1	6.5	P14	8.0	EH44	7.0	EH26	7.3	S
EH21	6.5	EH20	8.0	EH26	7.0	P27	7.0	S
P11	6.5	P77	8.0	EH25	6.5	EH44	7.0	S
		EH26	7.5	EH32	6.5	P067	7.0	S
		EH18	7.5	EH36	6.5	EH7	7.0	S
		EH39	7.5	EH22	6.5	EH6	6.8	S
		EH21	7.5	EH2	6.5	EH21	6.7	S
		EH25	7.0			EH3	6.7	S
		S62	6.5			EH25	6.2	S
		EH6	6.5					S
		EH4	6.5					S
EH38	6.0	EH35	6.0	EH39	6.0	EH39	6.0	MS
EH27	6.0	EH9	6.0	EH18	6.0	EH28	6.0	MS
EH28	6.0	P27	6.0	EH33	6.0	EH22	6.0	MS
EH42	6.0	EH33	6.0	EH21	6.0	EH33	6.0	MS
Ken/MP79	5.5	P57	6.0	EH8	6.0	EH38	5.8	MS
EH7	5.5	EH27	5.5	EH6	6.0	P57	5.8	MS
		EH30	5.5	EH35	5.5	P11	5.8	MS
		S63	5.5	EH45	5.5	EH18	5.7	MS
		EH31	5.5			EH45	5.5	MS
						Ken/MP79	5.5	MS
						EH30	5.3	MS
						EH27	5.3	MS
						S62	5.3	MS
EH9	5.0	EH16	5.0	EH3	5.0	S63	5.0	MR
MP63/P11	5.0	EH1	5.0	EH42	4.5	MP63/P11	5.0	MR
S63	5.0	P11	5.0	S63	4.5	EH42	5.0	MR
EH25	5.0	EH11	5.0	P57	4.5	EH35	5.0	MR
EH39	4.5	EH13	5.0	EH19	4.5	EH1	4.8	MR
		EH19	5.0	EH27	4.5	EH9	4.8	MR
		EH24	5.0	EH37	4.5	EH19	4.3	MR
		EH38	4.5			EH37	4.3	MR
		EH32	4.5			EH4	4.2	MR
		EH15	4.5			EH32	4.2	MR
		EH42	4.5					MR
		EH37	4.5					MR
EH37	4.0	Z55	3.5	EH28	4.0	EH24	3.7	R
S62	4.0	EH14	3.5	N3/MP72	3.5	Z55	3.5	R
EH22	3.5	EH10	3.5	EH23	3.5	N3/MP72	3.5	R
EH18	3.5	EH12	3.0	EH24	3.5	MP75/E6	3.5	R
EH35	3.5	S71	3.0	EH4	3.5	EH16	3.3	R
MP75/E6	3.5	EH41	2.5	EH9	3.5	EH11	3.3	R
EH19	3.5	EH23	2.5	EH13	3.5	EH31	3.3	R
AF1	3.0	EH40	2.5	EH41	3.0	EH15	3.2	R
EH40	3.0	EH29	2.5	EH16	3.0	EH13	3.2	R
EH30	3.0	EH17	2.0	EH15	3.0	EH23	3.0	R
EH23	3.0	EH34	2.0	EH1	3.0	AF1	3.0	R
EH11	3.0			EH29	2.5	EH14	2.7	R
EH24	2.5			EH14	2.5	EH40	2.7	R
EH4	2.5			EH40	2.5	S71	2.7	R
P77	2.5			EH10	2.5	EH10	2.5	R
S71	2.5			EH31	2.5	Ken/MP34	2.5	R
Ken/MP34	2.5			S71	2.5	EH41	2.5	R
EH16	2.0			EH11	2.0	P77	2.5	R
EH41	2.0			EH34	1.5	EH29	2.3	R
EH29	2.0			EH17	1.5	EH12	1.8	R
EH31	2.0			EH12	1.0	EH34	1.7	R
EH14	2.0					EH17	1.5	R
EH15	2.0							R
EH32	1.5							R
EH34	1.5							R
EH10	1.5							R
EH12	1.5							R
EH17	1.0							R
EH13	1.0							R
Mean	4.4		5.7		5.0		5.0	
CV (%)	24.8		16.6		18.1		20.0	
LSD (0.05)	2.2		1.9		1.8		1.3	

†C108 = Cedara, November 2007 planting, C208 = January 2008 planting, C09 = Cedara, November 2008 planting. ‡Disease reactions: R = resistant, MR = moderately resistant, S = susceptible and MS = moderately susceptible. Pannar brand = P, SeedCo brand = S, Experimental hybrids = EH
Crosses with MP coded lines are also experimental hybrids

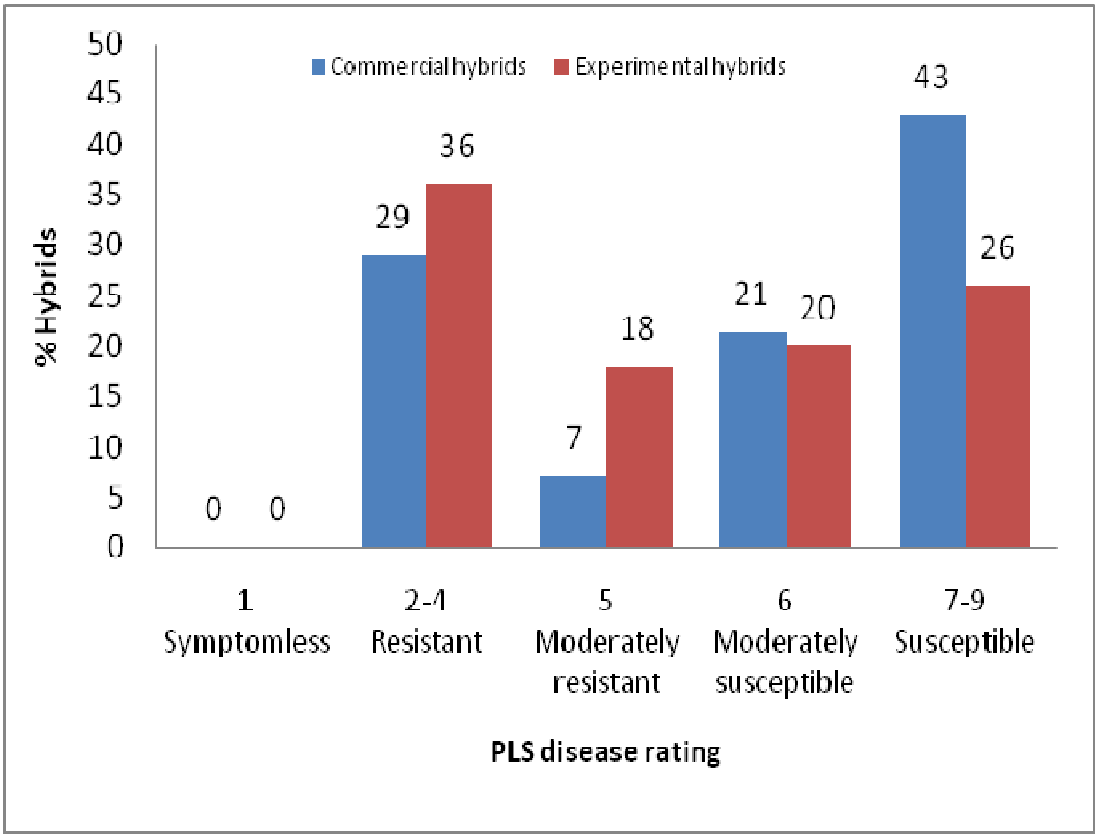

Figure 3.2 Frequency distribution of mean Phaeosphaeria leaf spot ratings of the hybrids evaluated over three seasons at Cedara Agricultural Research Station

3.5.3 Area under disease progress curves for disease severity scores

Analysis of variance for area under disease progress curves are presented in Table 3.7. Mean squares for genotypes, environment and genotype x environment interaction were all highly significant (P≤0.001) for PLS SAUDPC. The SAUDPC values in the two seasons were variable with values ranging from 1.0 to 6.1 in 2007/8 season and from 0.8 to 6.4 in 2008/9 season (Table 3.8). Overall, the combined 2-year data for SAUDPC were significantly different (P=0.05). Generally SAUDPC values were higher in 2008/9 than in 2007/8 season (Table 3.8). The lines with higher mean PLS disease scores had higher SAUDPC values and those with lower PLS scores had lower SAUDPC values. Correlation coefficients between SAUDPC values for disease severity scores with the final PLS scores in both seasons (Table 3.9) were highly significant (P≤0.001) and positive (r ≈ 0.92-0.97), irrespective of the anthesis grouping. In both seasons, the SAUDPC for disease severity scores and final PLS scores were significant (P≤0.001) and positively correlated (r ≈ 0.61 and 0.73) with 50% to anthesis for the early flowering group (67-74 days). For the other two groups with anthesis above 74 days, the correlation between anthesis and final PLS scores and SUDPC for disease severity scores were not

significant (P>0.05). The final The inbreds MP70, CM34, CM35, Early34, CIM58, CIM59 and the farmers' collections (NTL8s, DLs and KLs) had higher SAUDPC values than the rest of the lines and populations. Some populations were highly variable in their SAUDPC values in the two seasons. For example, QM7 and QM6 had lower SAUDPC (1.2 and 2.5 respectively) in 2007/8 season than in the 2008/9 season.

Table 3.7 Analysis of variance of SAUDPC for severity scores from two seasons (2007/8 and 2008/9) and combined data over the two seasons

	2007/8		2008/9		Combined	
Source	DF	MS	DF	MS	DF	MS
Replication (Rep)	1	0.31	1	1.68		
Genotype	70	4.07***	57	6.39***	72	8.03***
Error	72	0.36	81	0.55	155	0.47
Environment (Env)					1	12.69***
Rep/Env					2	1.30
Genotype*Env					55	2.01***
Corrected Total	143		139		285	
CV (%)	23.94		25.49		25.11	
Mean	2.50		2.92		2.71	

Table 3.8 SAUDPC for PLS disease severity scores and incidence of inbreds and populations from two seasons (2007/8 and 2008/9) and combined data over the two seasons

			SAUDPC for PLS Disease Severity score			SAUDPC for PLS Incidence	
Genotype	Mean PLS score	Disease Reaction	2007/8	2008/9	Combined	Mean %Incidence	2008/9
MP70	9.0	S	6.1	6.4	6.2	100.0	7.4
CM35	9.0	S	5.8	6.4	6.1	95.0	7.2
CM34	8.8	S	5.8	5.6	5.7	100.0	7.3
NTL8-1	8.5	S	4.9	6.0	5.4	87.0	6.5
KL-1	8.5	S	4.8	5.4	5.1	93.0	6.6
NTL8-4	8.3	S	4.6	6.4	5.5	84.0	6.7
NTL8-2	8.3	S	5.0	5.7	5.3	66.0	5.8
P12	8.0	S	5.2	4.5	4.9	84.0	6.2
KL-2	8.0	S	4.2	5.7	5.0	86.0	6.3
KL-3	8.0	S	5.1	5.2	5.1	82.0	6.4
MP58	8.0	S	3.5		3.5		
Early34	7.8	S	2.9	3.6	3.3	86.0	5.6
NTL8-3	7.5	S	3.4	4.3	3.9	73.0	5.7
DL-2	7.5	S	3.6	5.3	4.4	80.0	6.2
DL-1	7.5	S	3.5	5.0	4.3	69.0	5.8
CIM58	7.5	S	3.6	4.2	3.9	97.0	7.1
CIM59	7.0	S	3.5	3.6	3.5	87.0	6.4
P5	7.0	S	3.1		3.1		
DL-3	6.5	S	3.5	4.8	4.1	75.0	6.3
T4	6.0	MS	3.1	3.9	3.5	78.0	6.3
Kenyan	5.8	MS	2.3	4.3	3.3	35.0	4.3
CZL00009	5.5	MS	3.5	2.3	2.7	64.0	5.1
CML441	3.0	MS	2.7	2,3	2.42	64.0	5.1
QM6	5.3	MS	2.5	4.7	3.6	44.0	4.6
CZL00001	5.3	MS	2.1	2.7	2.5	91.0	4.9
CML205	5.2	MS	2.8	4.1	3.7	72.0	5.7
E6	5.0	MR	2.0		2.0		
MP59	5.0	MR	3.4		3.4		
QM7	4.8	MR	1.2	5.5	3.3	89.0	6.3
NAW5885	4.5	MR	3.0	2.2	2.6	43.0	3.8
CML443	4.5	MR	2.1	3.2	2.8	69.0	5.1
MP66	4.3	MR	2.1	1.9	2.0	58.0	4.6
CML312	3.8	R	2.6	2.0	2.2	48.0	3.3
A1220-4	3.8	R	3.4	2.1	2.5	23.0	3.4
QM3	3.8	R	2.4	2.7	2.6	59.0	4.9
CML504	3.5	R	1.5	2.4	1.9	73.0	5.0
A13	3.5	R	1.4	2.9	2.1	58.0	4.8
CZL03003	3.5	R	3.9		3.9		
CML373	3.5	R	1.7	2.3	2.0	45.0	4.2
P6	3.5	R	1.5		1.5		
PN7(T2)	3.3	R	1.8	1.9	1.8	11.0	1.6
CML445	3.2	R	2.2	1.7	1.9	42.0	2.8
A15	3.0	R	1.8	2.3	2.1	33.0	3.6
N2109	2.8	R	4.4	3.4	3.9	41.0	4.5
CZL00003	2.8	R	1.1	1.8	1.5	51.0	3.3
CML312P	2.8	R	1.2	2.0	1.6	28.0	2.8
CML442-B	2.5	R	3.3	2.3	2.8	55.0	4.9
MP13	2.5	R	1.3		1.3		
CML489	2.5	R	1.5	2.0	1.7	19.0	3.1
MP18	2.3	R	2.4	1.7	2.0	63.0	3.2
CML202	2.3	R	1.3	1.6	1.4	51.0	2.9
P20	2.3	R	1.1	1.5	1.3	30.0	2.1
N3	2.2	R	1.1	1.6	1.4	13.0	1.9
B18	2.0	R	1.2		1.2		
CML440	2.0	R	1.3	1.6	1.4	28.0	2.6
CZL01002	2.0	R	1.3		1.3		
CML488	1.8	R	1.3	1.4	1.4	32.0	3.1
P3	1.8	R	1.3	1.2	1.2	14.0	1.6
MP12	1.8	R	1.2	0.8	1.0	98.0	5.5
B17	1.8	R	1.4	1.5	1.5	18.0	2.3
CML444	1.5	R	1.0	1.6	1.3	31.0	3.6
MP78	1.5	R	1.1	1.1	1.1	9.0	1.1
QM5	1.5	R	1.2		1.2		
CM31	1.5	R	1.0	1.8	1.4	21.0	2.6
PN8(T3)	1.3	R	1.2	1.4	1.3	9.0	1.8
CZL00018	1.3	R	1.1	1.2	1.1	5.0	1.0
MP5	1.0	Symptomless	1.0	1.0	1.0	0.0	0.7
MP1	1.0	Symptomless	1.0		1.0		
CZL00034	1.0	Symptomless	1.0		1.0		
CM33	1.0	Symptomless	1.0	1.0	1.0	0.0	0.7
Mean	**4.2**		**2.5**	**2.9**	**2.8**	**53.2**	**4.2**
CV (%)	**28.6**		**24.0**	**25.5**	**25.1**	**33.9**	**24.5**
LSD (0.05)	**1.8**		**1.2**	**1.4**	**1.0**	**34.7**	**2.0**

3.5.4 Area under disease progress curves for PLS disease incidence

The disease incidences for the inbreds and populations observed in 2008/9 season are presented in Table 3.8. The mean incidence values ranged from 0 to 100% and the differences were significant (P = 0.05). In general, the susceptible lines and populations had higher incidences. However, there were some inbreds or populations which had resistant reactions but had high disease incidences. These included; QM7, CML443, MP66, MP12, CML504, QM3, and A13 and their incidences ranged from above 50 to 98%. The mean disease incidence by the end of the season was about 53%. Lines and populations which had high PLS incidences also had high SAUDPC values (Table 3.8).

Table 3.9 Pearson correlation coefficients among SAUDPC values, final PLS scores and days to flowering for the inbreds evaluated in 2007/8 (above diagonal) and 2008/9 (below diagonal) seasons

	50 days to Anthesis	50 days to Silking	SAUDPC_Score	PLS_Score
Inbreds with 67-74 days anthesis				
50 days to Anthesis	1	0.56^{NS}	0.79*	0.85**
50 days to Silking	-0.14^{NS}	1	0.73^{NS}	0.65^{NS}
SAUDPC_Score	0.61*	-0.30^{NS}	1	0.97***
PLS_Score	0.73***	-0.26^{NS}	0.95***	1
Inbreds with 75 -81 days anthesis				
50 days to Anthesis	1	0.62***	-0.17^{NS}	-0.24^{NS}
50 days to Silking	0.47*	1	-0.47*	-0.53**
SAUDPC_Score	0.059^{NS}	-0.47*	1	0.95***
PLS_Score	0.096^{NS}	-0.48*	0.85***	**1**
Inbreds with anthesis over 81 days				
50 days to Anthesis	1	0.91***	-0.18^{NS}	-0.12^{NS}
50 days to Silking	0.76***	1	-0.12	-0.05^{NS}
SAUDPC_Score	0.019^{NS}	-0.038^{NS}	1	0.92***
PLS_Score	0.003^{NS}	0.006^{NS}	0.94***	**1**

*, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001

3.5.5 Disease progress curves for PLS disease severity and incidence

A selection of disease progress curves representative of the range of disease values observed in the field in the two seasons are shown in Fig.3.3 and 3.4. There were some marked differences in the severity of the disease epidemics caused by PLS in the inbreds and populations. In 2008/9 disease appeared much earlier, with some lines and populations showing symptoms as early as 67 days after planting (data not shown). In 2007/8 season disease was noticed 83 days after planting (DAP). The progress of the disease in 2007/8 season was slow at first; with a prolonged lag phase after appearance of first disease symptoms between 83 and 104 DAP for most of the lines and populations. Thereafter, there was a substantial increase from 104 to 142 DAP in most of the susceptible lines and populations such as NTL8-1, Early34 and CIM58. However, the most susceptible line, MP70 had a logarithmic increase right from the onset of the disease 83 DAP, with the rate slowing down from 126 days. In 2008/9 season the progress of the disease was different from 2007/8 season. There was no distinct lag phase for most of the lines and populations. The increase was gradual for most of the lines and it levelled off from 138 DAP.

Progress of PLS disease incidence in 2008/9 is presented in Fig 3.5. By 91 DAP; the most susceptible genotypes had incidences ranging from about 40 % to slightly less than 100%. The most susceptible genotype, MP70 had more than 90% disease incidence by 91 DAP. The most susceptible genotypes MP70 and NTL8-1 reached 100% disease incidence by 105 DAP. The genotypes also differed in the disease incidence progress. Some showed gradual increments, others sharp increases such as Early34 which increased from 20% at 98 DAP to almost 70% by 112 DAP. The disease increases for most of the genotypes levelled off from 123 DAP, whilst others like CML202 increased from 123 to 147 DAP. Overall, the disease incidences ranged from 30% to 100% at the end of the season. The resistant to moderately resistant genotypes had lower incidences than the most susceptible genotypes.

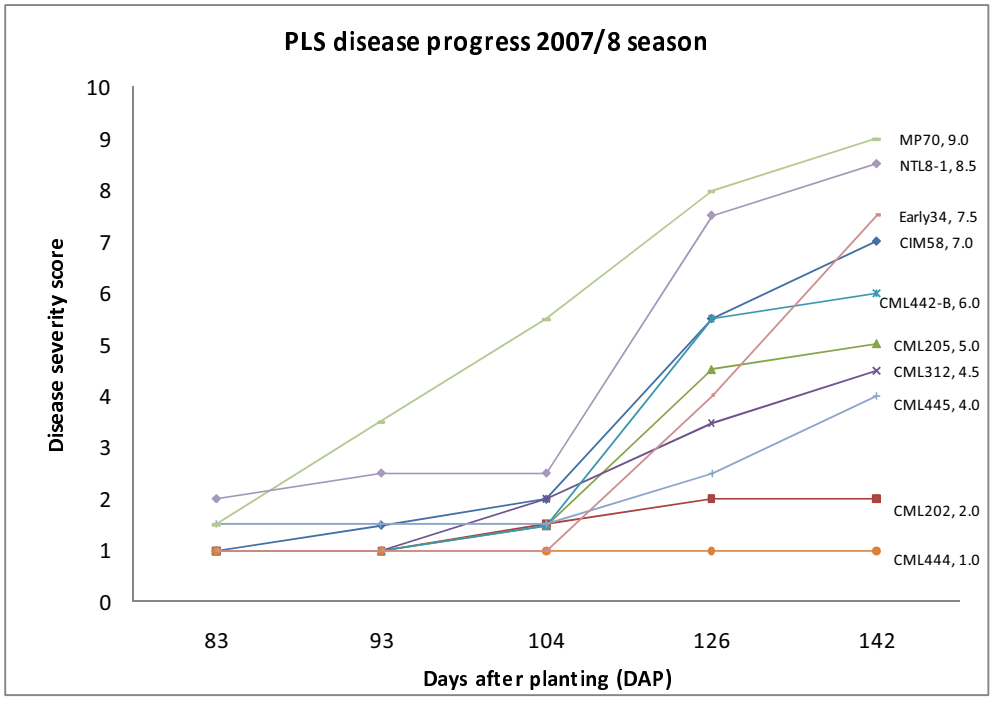

Figure 3.3 Disease progress (severity scores) of ten selected lines and populations representative of the range of values observed in the field in 2007/8 season at Cedara Agricultural Research Station

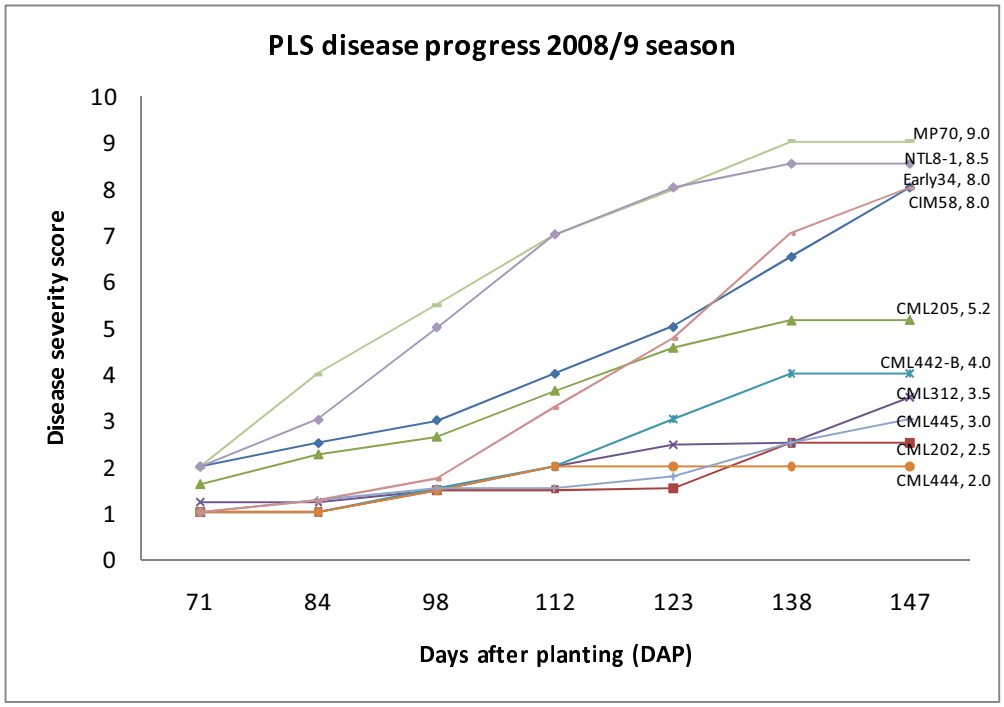

Figure 3.4 Disease progress (severity scores) of ten selected lines and populations representative of the range of values observed in the field in 2008/9 season at Cedara Agricultural Research Station

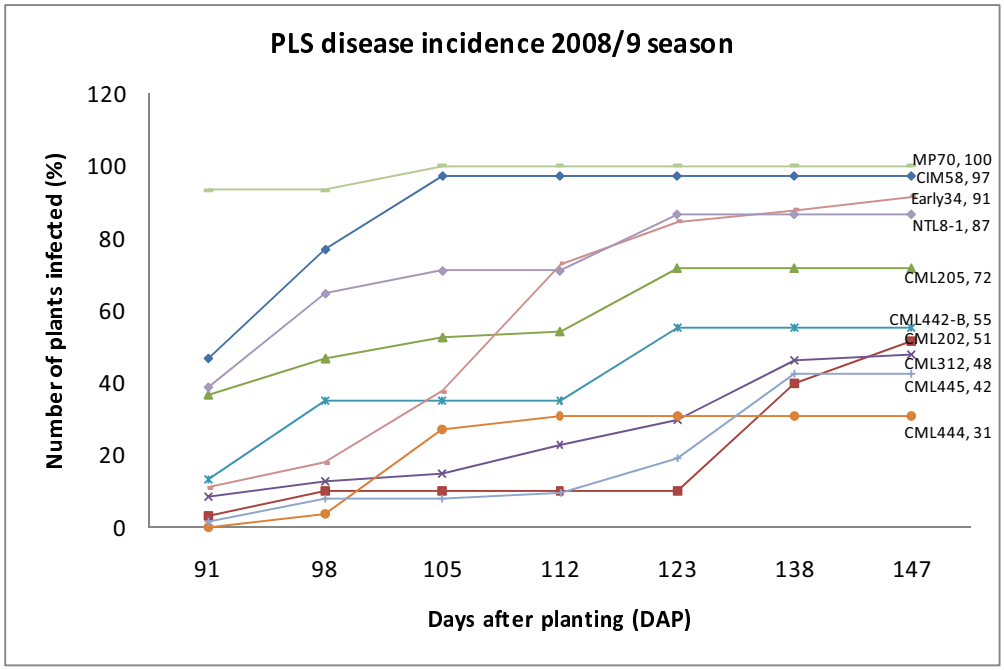

Figure 3.5 Disease progress (% incidence) of ten selected lines and populations representative of the range of values observed in the field in 2008/9 season at Cedara Agricultural Research Station

3.5.6 Variance components and broad-sense heritability estimates

Variance components and broad-sense heritability estimates are presented in Table 3.10. The heritability estimate was moderate (55%).

Table 3.10 Variance components and heritability estimates for the combined environments for PLS resistance

Inbreds	
Variance component	Combined
σ_e	1.89
σ_g	3.33
σ_{gxe}	0.79
σ_p	6.01
Broad sense heritability (%)	55.40

3.6 Discussion

Disease symptoms for PLS were noticed around flowering time. In 2007/8 the earliest lines flowered in about 70 DAP and symptoms were noticed around 76 DAP, while in 2008/9 season, the early lines flowered around 66 DAP and symptoms were noticed around 67 DAP (data not shown). Symptoms in most of the susceptible lines developed rapidly resulting in severe necrosis and great reduction in photosynthetic tissue. There was a negative correlation between flowering days and the final PLS disease severity scores and AUDPC values. Depending on the weather conditions and how the disease progresses, this early appearance of the disease has great potential to cause serious reductions in grain yield. For example in Brazil, a significant positive correlation between grain yield reduction and PLS disease severity was reported (Pegoraro *et al*., 2002).

The PLS incidence observed in 2008/9 season clearly indicated that the disease was present in high levels and the distribution was uniform. Incidences of 100% and high severity scores (75 to 100% leaf area showing disease) were observed especially in the susceptible lines, populations and hybrids. The incidence data indicated that inoculum at Cedara was by and large uniformly distributed with a small percentage of symptomless plants. The symptomless plants could have been either escapes or a resistant reaction. Therefore, as a screening site, Cedara Agricultural Research Station was a reliable spot and the differences observed were in general as a result of genetic differences. Derera *et al.* (2007) also reported high PLS disease pressure at Cedara Agricultural Research Station. Generally disease development was from the bottom leaves upwards in most of the genotypes. However, there were some genotypes like Early34, which developed symptoms from the top leaves downwards. Disease progress for this line was initially slow, and then it increased sharply from about 98 DAP in both seasons (Fig 3.3 and 3.4). This observation showed that PLS disease inoculum was coming from the soil, plant debris as well as airborne, an observation which was in agreement with reports by Derera *et al*. (2007) on PLS inoculum.

Disease development was variable from season to season, resulting in different levels of PLS severity. This contributed to the significant genotype x environment interaction (GEI) that was observed in both the inbreds and hybrids. There was a change in the disease reaction and ranking for some of these genotypes, for example Early34. For Early34, it is probably because the disease started from the top much later in the season and the spread could have been affected by the prevailing weather conditions at the end of the season. The inbred line CML441 had scores of 6.0 and 7.0 in C07 and C08, respectively,

and a score of 2.0 in C09. In 2008/9 season (C09) CML441 was affected by NLB (data not shown). The NLB disease appeared much earlier in the season (that is, before flowering) and increased in severity, colonizing most of the tissue by the flowering time, leaving very little for PLS to infect. This resulted in the lower PLS score recorded in C09 than C07 and C08. Observations of lines behaving differently to PLS infection in different seasons were also reported by Carson (1999), where some lines rated about 4.0 or 4.5 in one season and 1.0 in the other season. Smit and Lawrance (2004) also observed a significant GEI for PLS disease evaluation in different seasons. They attributed most of this interaction to the variation in PLS severity and prevailing weather conditions during the four seasons of their study. Overall, GEI was shown to be of minor importance in PLS resistance (Carson, 2001; Carson *et al*., 2005).

Significant variation was observed among the inbreds, populations and hybrids. Overall, about 58% of the inbreds/populations were resistant or moderately resistant to PLS. Many of these inbreds and populations are therefore potential sources of PLS resistance. Inbred lines like SC and N3 which are important in the region and CIMMYT's most successful lines such as CML395, CML444, CML202, CML312, and CML488 were resistant. Some lines like CML441 and P5, which were derivatives of ZM605, were also susceptible. ZM605 is a highly productive synthetic population from mid-altitude environments in Africa. Therefore these inbred lines need to be improved for PLS resistance.

Fifty-four percent of the single cross experimental hybrids and 46% of the commercial hybrids were resistant to PLS. This high level of resistance was in agreement with observations made by other researchers, who reported high levels of resistance in the single cross experimental hybrids or commercial hybrids (Carson, 1999; Flett and Lawrance 2004; Derera *et al*., 2007). This high level of resistance in the single cross hybrids could be attributed to the mode of resistance of PLS. Resistance to PLS was shown to be controlled mainly by additive gene action and was partially dominant (Carson, 2001; Silva and Moro, 2004; Mhembere, 2005; Derera *et al*., 2007; Vivek *et al*., 2009). However, non-additive gene action was also important. Some of the resistant hybrids observed in this study involved crosses between susceptible and resistant parents. This result is in agreement with the observation made by Carson (2001) where susceptible parents crossed with resistant parents produced resistant hybrids. These R x S crosses also confirmed that resistance to PLS in the inbred lines used was predominantly controlled by genes with additive effects. Therefore, it is possible to use PLS susceptible parents crossed with resistant parents to produce resistant hybrids. This

implied that progress in PLS disease resistance could be made through selection. However, 64% of the commercial hybrid checks evaluated were susceptible to PLS. This suggested the need to improve the parents that make up these hybrids for PLS disease resistance through backcrossing or develop new hybrids with high levels of resistance to PLS.

The results also revealed more disease in the late planted crop at Cedara (C208) compared to early season's plantings. This result was supported by the observation made in Brazil where severe infestations occurred in late plantings (Fernandes, 1998; Cervelatti *et al*., 2002). Increase in PLS disease incidence has been attributed mainly to practices such as late planting, absence of rotation, and zero tillage practices (Casela, 1998; Cervelatti *et al*., 2002).

AUDPC reflects the disease progress through the season. This helps to capture changes that take place during the season in the environment. Correlation coefficients between AUDPC values for disease severity with PLS final severity scores in both seasons were significant and positive. This implied that the ranking of genotypes for AUDPC and final PLS disease severity scores were generally similar. This meant that a single assessment for the final disease severity would be adequate, especially for screening large numbers of germplasm. This would be less laborious than the several assessments required to obtain AUDPC values. Freppon *et al*. (1996) and Saghai-Maroof *et al*. (1996) reported similar results with grey leaf spot disease (GLS) of maize, where a single assessment was as effective in identifying resistant germplasm as multiple assessments. The assessment would be more significant when done at or near the peak of the epiphytotic. In both seasons, the AUDPC for disease severity were significant and positively correlated with 50% to anthesis for the early-flowering group (67-74 days). This implied that for the early flowering lines, more disease developed and the SAUDPC values were high. Since the disease symptoms were first observed around flowering, and the weather conditions were still favourable, more disease thus developed on the susceptible early varieties. No significant correlation was observed for anthesis with final PLS score and SAUDPC for the other two groups (medium and late flowering groups), which suggests that the resistance level was not influenced by maturity in these groups.

Although PLS disease was observed after flowering, in the most susceptible genotypes the disease progress was rapid. However, in some of the lines the severity only increased towards the end of the season. A similar trend to this observation was reported in Brazil where PLS was initially seen at the end of the season thus not causing any major

damages to the maize quality or grain yield (Silva and Moro, 2004). However, in Brazil, with time inoculum started building up over the seasons resulting in significant damage on maize and grain yield reductions of more than 60% in susceptible cultivars (Cervelatti *et al*., 2002). This implies, therefore, that although PLS appears not to be causing any significant yield losses in the region it has the potential of causing serious damage as build-up of inoculum continues season after season.

Broad-sense heritability estimates based on the inbred lines used was moderate for the combined environments. The broad-sense heritability estimate was within the range of heritability estimates reported for PLS in other studies involving different germplasm (Carson, 2001; Mhembere, 2005; Derera *et al*., 2007). Differences in heritability estimates amongst different studies could be a result of differences in either the environment and/or the genotypes used, or GEI as well as different levels of additive variance versus dominance and epistatic variances as suggested by Falconer and Mackay (1996). The moderate heritability estimates imply that progress in PLS resistance can be made through mass selection.

3.7 Conclusion

Overall, about 58% of the inbreds/populations were resistant or moderately resistant to PLS. Some inbred lines like SC and N3 which are significantly important in the region and the historically successful CIMMYT lines which included CML395, CML444, CML202, CML312, and CML488 were resistant to PLS. There was a high level of resistance in the single cross experimental hybrids. However, 64% of the commercial hybrid checks evaluated were susceptible to PLS, suggesting the need to improve the parents for PLS disease resistance or to develop new hybrids with high levels of resistance to PLS. Correlation coefficients between AUDPC values for disease severity with PLS final severity scores in both seasons were significant and positive resulting in similar rankings of the genotypes. Therefore, a single assessment for the final disease severity would be adequate for PLS resistance, especially for screening large numbers of germplasm. The AUDPC for disease severity were significant and negatively correlated with flowering days (50% to anthesis and silking), suggesting that the early flowering lines get more disease resulting in higher AUDPC values. Broad-sense heritability estimates varied from moderate to high, showing that phenotypic selection for PLS resistance would be effective as a breeding strategy. The results have demonstrated that high levels of resistance are available in the regionally adapted germplasm and additional sources of resistance to PLS have been identified. Some of the experimental hybrids that exhibited high levels of resistance are recommended for further testing and release.

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4 Combining Ability Analysis for Phaeosphaeria Leaf Spot Resistance and Agronomic Traits in Tropical Advanced Maize Inbred Lines

Abstract

Although Phaeosphaeria leaf spot (PLS) of maize is increasing in importance in sub-Saharan Africa (SSA), there is still limited information on the combining ability for disease resistance of the germplasm that are adapted to African environments. Evaluating combining ability effects and their interactions with the environment would provide valuable information that can be used in the development of cultivars that are resistant to PLS. This study was therefore conducted to determine the combining ability, gene action and heterosis estimates for resistance to PLS among selected tropical advanced maize inbred lines. Forty five F_1 hybrids were generated by crossing ten inbred lines in a half diallel mating scheme. The 45 hybrids along with the ten inbred parents were evaluated in four environments, with two replications each between 2007 and 2009. General combining ability (GCA) and specific combining ability (SCA) effects were highly significant ($P \leq 0.001$) for PLS, grain yield and all the other agronomic traits. GCA effects accounted for 90% and SCA effects 10% of the variation in the hybrids for PLS resistance, whereas GCA effects for grain yield and the other agronomic traits measured, accounted for 65-87% of the variation. This indicated the predominance of additive over non-additive gene action for all the traits in these inbred lines. The most resistant inbred lines to PLS were A1220-4, N3, A16, MP18 and CML488. These lines had good combining ability for PLS resistance and contributed towards resistance in their crosses. In general, resistant hybrids involved a susceptible and a resistant parent, whereby at least one of the parents had a negative GCA effect. In addition, lines A1220-4 and A16 contributed towards high yield and were late maturing. Inbred line CZL00009 conferred genes for early maturity. By and large, highly significant additive gene action implied that progress would be made through selection, although the significant non-additive gene action could slow progress. The study also revealed that the use of one parent with resistance would provide adequate PLS resistance in single cross hybrids. Therefore dominance effects which were associated with reduced disease levels may be exploited in developing single cross maize hybrids among these inbreds when one of the parents is resistant.

4.1 Introduction

Phaeosphaeria leaf spot (PLS) is a fairly new disease in Africa. It is caused by the ascomycete fungus *Phaeosphaeria maydis* (Henn.) Rane, Payak & Renfro (syn. = *Leptosphaeria zeae-maydis* Saccas; *Metasphaeria maydis* (Henn.) Höhnel). Although not mentioned in the available literature among the dominant constraints limiting maize yields in sub-Saharan Africa (SSA), the disease has great potential to threaten regional food security. Yield losses of more than 60% have been observed in countries such as Brazil, where the disease is of major economic importance (Cervelatti *et al*., 2002). In South Africa, Kenya, and Zimbabwe, PLS disease incidence has increased since the early 1990s (Mwangi, 1998; Smit and Lawrence, 2004; Mhembere, 2005). For example, in Kenya incidences of over 85% were recorded for PLS diseases in some districts (Mwangi, 1998).

Currently, no specific control measures have been reported for PLS disease, resulting in great concerns from farmers and breeders. In South Africa, curative control using fungicides including those that control maize grey leaf spot (GLS) disease, has not been effective (Flett and Lawrance, 2004). Resistant cultivars would therefore be more sustainable and effective as a control measure for increased maize yields in all the farming sectors.

Preliminary evaluations for PLS resistance in South Africa and Zimbabwe have indicated cultivar variation (Flett and Lawrence, 2004; Mhembere, 2005). This implies that development of inbreds with adequate levels of resistance to PLS would be possible. Derera *et al*. (2007) identified lines that contributed exceptionally high resistance to PLS which could be used as breeding sources. However, information is still limited on the combining ability and mode of resistance to PLS in most of the germplasm that are adapted to African environments. Identification of more sources of resistance especially from heterotic groups that are adapted to subtropical environments and are widely used in breeding programmes in Africa would be valuable. Additionally, evaluating heterotic and combining ability effects and their interactions with the environment would provide valuable information that can be used in the development of cultivars that are resistant to PLS. Therefore, this study was conducted to: i) estimate the combining ability effects for resistance to PLS among selected maize inbred lines, ii) determine the gene action controlling PLS resistance, and iii) estimate heterosis on PLS resistance and grain yield in tropical African maize lines and their crosses.

4.2 Materials and Methods

4.2.1 Maize Germplasm

Maize inbred lines were obtained from the CIMMYT programme in Harare, Zimbabwe, while the N3 inbred was obtained from the Crop Breeding Institute in Zimbabwe. The inbred lines were sampled from the major heterotic groups that are adapted to subtropical environments and are indicated in Table 4.1. The heterotic groups included the CIMMYT A and B classification (CIMMYT, 2001), the SC (Southern Cross), N3 (derived from Salisbury white), and the "P" heterotic group (derivatives from Natal Potchefstroom Pearl) (Gevers and Whythe, 1987; Olver, 1998; Derera, 2005). The CIMMYT group A is mainly derived from populations such as the Tuxpeno, Kitale, BSSS (Iowa Stiff Stalk Synthetic), B73 and N3, whereas CIMMYT group B are derivatives from populations such as the ETO, Ecuador 573, Lancaster, Mo17 and SC (CIMMYT, 2001).

Table 4.1 Designation, pedigrees and heterotic groups for parent inbred lines used in the diallel analysis

Designation†	Pedigree or Population (OPVs)	Heterotic grouping	Selection criteria
A1220-4	[(CML395/CML444)-B-4-1-3-1-B/CML395//SC/ZM605#b-19-2-X]-1-2-X-1-1-BBBBBB]-7-1-3-2-BBB	B / SC	PLS susceptible
CML205	[EMSR]#B#bF101sr-2-1-sr-3-2-4-b-b	B	GLS susceptible
A16	Original pedigree CML312 (S89500F2-2-2-1-1-B*5)	A	GLS susceptible
CML445	[[TUXPSEQ]C1F2/P49-SR]F2-45-7-5-1-BBB	AB	PLS susceptible
CML488	DTPWC8F31-4-2-1-5-BBB	AB	PLS resistant
CZL00001	INTA-191-2-1-2-BBBB	A	PLS susceptible
CZL00009	INTA-F2-192-2-1-1-1-BBBBB	A	PLS susceptible
MP18	[[[NAW5867/P30SR]-111-2/[NAW5867/P30SR]-25-1]-9-2-3-B-2-B/CML388]-B- 35-2-B-1-#-1-BB	A / P	GLS susceptible
N3	Salisbury White	N3	GLS susceptible
CML443	[AC8342/IKENNE{1}8149SR//PL9A]C1F1-500-4-X-1-1-BB-1-BB	AB	GLS resistant

†some of the lines like A1220-4, A16 and MP18 were coded for convenience of study.

Maize lines with white grain colour only were used. Some of the inbred lines were derived from lines such as CML395, CML444, CML206, SC and N3 which form the basis of most productive hybrids in medium to high altitude environments. Other lines were derived from ZM605 which is a highly productive synthetic population for mid-altitude environments in Africa. Where known, the lines were further selected based on their reactions to PLS and grey leaf spot (GLS) diseases from previous studies and to other abiotic stresses.

4.2.2 Field Evaluations

Ten advanced maize inbred lines were crossed in a half diallel mating scheme. The resulting 45 single cross F_1 hybrids plus nine standard checks were evaluated in 2007/8 and 2008/9 seasons in a total of four environments. The parents were also evaluated in trials adjacent to the hybrid trials, but only in two environments. Two locations were used for this study and these were: Cedara (C), South Africa (30°16'E, 29°32'S, 1130 metres above sea level (m.a.s.l) and Mpongwe, Zambia (ZAMB) (28°8'E, 13°31'S, 1219 m.a.s.l). At Cedara, plantings were done in November 2007 (C108), January 2008 (C208) and November 2008 (C09), while at Mpongwe, planting was done in January 2008 (ZAMB08). The F_1 hybrids and standard hybrid checks were laid out in the field in two replications using a 9 x 6 alpha (0, 1) lattice design in each environment. Inbred parents were planted in a 10 x 2 randomised complete block design with two replications, on the same day as the hybrids. The plot size for both the hybrids and parental lines in each environment was two rows, 3 m long, with 0.75 m inter-row spacing and 0.3 m intra-row spacing, except for Mpongwe where plots were one row, 5 m long with 0.75 m between rows and 0.3 m between the plants. Plant population densities were about 44 000 per hectare in all the four environments.

A susceptible maize hybrid and inbred line were used as borders for the hybrid and inbred trials, respectively. Fertiliser was applied at the rate of 120 kg N, 33 kg P, and 44 kg K. Standard cultural practices including ploughing and disking, hand planting, hand weeding and/or application of herbicides and fertilizers were followed at each site.

4.2.3 Disease assessment

Phaeosphaeria leaf spot (PLS) disease severity was assessed fortnightly from the first appearance of symptoms, based on visual assessment of the whole plot using a 1-9 logarithmic rating scale, where 1 = 0% , 2 = <1%, 3 = 1-3%, 4 = 4-6%, 5 = 7-12%, 6 = 13-25%, 7 = 26-50%, 8 = 51-75% and 9 = 75-100% leaf area showing disease symptoms. The scores were further classified into the following disease reaction types; 1.0 = symptomless, 2.0-4.0 = resistant, 4.1-5.0 = moderately resistant, 5.1-6.0 = moderately susceptible, 6.1-9.0 = susceptible. The score recorded at the hard-dough stage was used for statistical analysis. Other agronomic traits measured included the number of days to mid-silking and pollen shed, plant and ear height following the standard practice used at CIMMYT (CIMMYT, 1985). At harvest grain yield was measured on a whole plot basis and adjusted to 12.5% moisture (Zimbabwe Marketing Standards) using the formula:

Grain Yield (t ha^{-1}) = [Grain Weight (kg/plot) x 10 x (100-MC)/ (100-12.5)/(Plot

Area)], where MC = Grain Moisture Content.

4.2.4 Data analysis

Mean disease ratings taken at the hard-dough stage of maize, grain yield and means of the other traits for the hybrids and standard checks were analysed using PROC GLM procedure in SAS 9.1 (SAS Institute, 2002). The data were subjected to ANOVA firstly by environment as the main effect, then a combined analysis across environments was conducted to analyse the effect of environments, hybrids and interactions. Data were analysed for combining ability using the Diallel SAS05 program in SAS (Zhang *et al*., 2005). Only the 45 experimental hybrids were used in the calculation of combining ability effects. The F_1 hybrids were treated as fixed effects in the statistical analysis and environments (both spatial and temporal environments) as random effects. The researcher had no control over the environment. This implied that interest was in selecting inbreds that would perform well on an average site through the years. To estimate the general combining ability (GCA) and specific combining ability (SCA) effects, Griffing's diallel analyses, Model 1 (fixed genotype effects), Method 4 (crosses only) was used according to the model:

$$Yijk = \mu + gi + gj + sij + eijk$$

Where*:*
Yijk = observed measurement for the ijth cross in the kth replication/ environment combination,
μ = overall mean,
gi and gj = GCA effects for the ith and jth parents, respectively,
sij = SCA effect for the ijth cross, and
eijk = error term associated with the ijth cross evaluated in the kth replication/environment combination.

The interaction terms were used to test for the significance of the corresponding main effects (Zhang and Kang, 1997). The environments and replications within environments were considered random and therefore tested against the residual error term.

Heterosis (H) for PLS and grain yield for each hybrid was estimated for the two environments that included parents, using mid-parent (MP) and better-parent (BP) scores (Falconer and Mackay, 1996) according to the following equations:

$$MPH\ (\%) = 100*(F_1\text{-}MP)/MP, \text{ and } BPH\ (\%) = 100*(F_1\text{-}BP)/BP$$

Where F_1 = mean of the F_1 hybrid performance, MP = mean of the two parents making the cross and BP = mean of the better parent (resistant or high yielding) in the cross.

4.3 Results

The results of the contrast of PLS severity scores and grain yield of the experimental hybrids versus checks are presented in Table 4.2. The entries (experimental hybrids plus checks), experimental hybrids, checks and their interactions with the environments were highly significant (P<0.001). The contrast of the PLS severity scores for the experimental hybrids against the checks was highly significant (P<0.001), but the interaction with the environment was not significant (P>0.05). The mean PLS severity scores for the experimental hybrids and checks were 4.5 and 5.7, respectively.

Table 4.2. Analysis of variance for PLS severity scores and grain yield: Experimental hybrids versus check hybrids.

		PLS		Grain Yield (t ha^{-1})	
Source	DF	Mean Square	F-Value	Mean Square	F Value
Environments(Env)	**3**	**85.90**	**105.48*****	**737.73**	**565.20*****
Rep(ENV)	**4**	**2.71**	**3.33****	**11.99**	**9.19*****
Entry	**53**	**26.01**	**31.94*****	**5.24**	**4.01*****
Experimental hybrids (Exp)	44	22.96	27.57***	5.27	3.85***
Checks (Chks)	8	34.59	44.08***	5.63	6.06***
Exp vs Chks	1	91.68	21.87***	0.46	0.21^{NS}
ENV*Entry	**159**	**1.99**	**2.44*****	**2.69**	**2.06*****
ENV*Exp	132	2.07	2.49***	2.46	1.79***
ENV*Chks	24	1.15	1.46^{NS}	2.30	2.48**
ENV*Exp vs Chks	3	5.08	1.21^{NS}	15.82	7.12***
Error	**212**	**0.81**		**1.31**	
Corrected Total	**431**				
Means					
Experimental hybrids	4.5			7.13	
Checks	5.7			7.22	

*, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001, respectively, NS = non-significant (P>0.05).

The entries (experimental hybrids plus checks) and their interaction with the environment were highly significant (P<0.001). The mean squares for experimental hybrids, checks and their interactions with the environments were also highly significant (P<0.001). The contrast of the grain yield for the experimental hybrids and checks was not significant (P>0.05). The mean grain yield for the experimental hybrids and checks were 7.13 t ha^{-1} and 7.22 t ha^{-1}.

4.3.1 Reaction of the inbreds and hybrids to PLS disease infection

Means of the parental lines, F1 hybrids and standard checks are presented in Table 4.3. and 4.4. There were significant (P≤0.05) differences among the hybrids for all the traits measured. The best performing experimental hybrids were not significantly different from the best commercial hybrid in terms of grain yield. The disease pressure was variable in the different environments as depicted by the different maximum scores recorded for both the inbreds (Table 4.3) and the F_1 hybrids (Table 4.4). The ten inbred lines (Table 4.3) used as parents in this study showed significant differences (P≤0.05) in their reaction to PLS.

Phaeosphaeria leaf spot means of the inbred parents in the two environments (C108 and C09) varied from 2.0 to 6.0. Inbred lines which were consistently symptomless to moderately resistant (scores 1.0 to 4.5) based on the classification used in this study included; N3, MP18, CML445, CML488 and A16. The most susceptible parent was CZL00009 with scores ranging from 5.0 to 7.0. The other inbreds had variable reactions in the three environments and these were; A1220-4, CML205, CML443 and CZL00001, with all four being moderately susceptible to susceptible in the different seasons at Cedara (C108 and C09).

There were significant differences (P≤0.05) amongst the F_1 hybrids for PLS disease in the different environments (Table 4.4). Disease pressure for PLS at Cedara for all the three plantings (C108, C09 and C208) was relatively high with maximum scores varying from 8.0 to 9.0. Most of the hybrids (about 80%) in the late planting (C208) had scores between 4.5 and 8.0 whereas for C108 and C09 the distribution was wider ranging from 1.0 to 8.5 and 9.0, respectively, with only 46 to 53% of the hybrids having scores between 4.5 and 9.0 (Table 4.4). In Mpongwe (ZAMB08), almost 70% of the hybrids had scores less than or equal to 4.0.

Table 4.3 Means of parental lines for disease scores and grain yield of maize across two environments

Entry	Genoytpes	PLS (1-9)	Type of Disease Reaction†	Grain Yield (t ha^{-1})
1	CML445	3.8	R	4.0
11	A1220-4	4.8	MR	2.2
20	CZL00009	6.0	S	4.0
28	CZL00001	5.3	MS	3.3
35	N3	2.3	R	5.4
41	CML205	5.3	MS	1.8
46	A16	4.0	R	1.7
50	MP18	3.5	R	2.0
53	CML443	4.5	MR	3.9
55	CML488	2.0	R	2.3
	Mean	4.2		3.1
	LSD (0.05)	1.2		2.0

†‡Disease reactions: R = resistant, MR = moderately resistant, S = susceptible and MS = moderately susceptible.

4.3.2 Grain Yield and other Agronomic Traits

There were significant differences (P≤0.05) in grain yield amongst the F_1 hybrids in the different environments (Table 4.5). Yields were relatively high in the normal seasons' plantings at Cedara (C108 and C09) and ZAMB08 ranging from about 4.0 to 13.0 t ha^{-1} compared to about 2.0 to 6.0 t ha^{-1} for the late planting at Cedara (C208). The commercial hybrid, S63 was amongst the highest yielding checks in C108, C09 and ZAMB08, but amongst the lowest in C208. Hybrids with ≥8.0 t ha^{-1} varied amongst the different environments, with C09 having almost 90%, C108, about 50% and ZAMB08 about 30% of the hybrids in that range.

Table 4.4 Reactions of the 45 F_1 hybrids and nine hybrid checks to Phaeosphaeria leaf spot disease infection tested over four environments between 2007 and 2009.

ENTRY	CED108†	CED09	ZAMB08	CED208	Across environments	Disease Reaction‡	Anthesis (days)
Hybrids with anthesis between 67 and 74 days							
CZL00009xCML205	7.0	8.0	4.0	8.0	6.8	S	71
CZL00009xMP18	3.5	6.5	5.5	8.0	5.9	S	72
CZL00009xCZL00001	3.5	6.0	5.0	7.5	5.5	MS	73
CZL00009xN3	3.5	4.5	3.0	5.0	4.0	R	73
CZL00009xCML488	3.0	3.5	2.0	2.5	2.8	R	72
A1220-4xCZL00009	1.5	2.5	1.5	3.5	2.3	R	74
Checks							
P17	8.5	9.0	7.0	8.0	8.1	S	74
S51	7.0	8.0	5.5	8.0	7.1	S	72
P27	8.0	7.0	5.0	6.0	6.5	S	73
15 most resistant hybrids with anthesis between 75 and 82 days							
A1220-4xCML488	1.0	1.5	1.5	2.0	1.5	R	77
A1220-4xN3	1.5	1.0	1.0	3.0	1.6	R	79
N3xCML488	1.5	1.5	2.5	2.0	1.9	R	76
CZL00001xCML488	2.0	2.5	1.5	2.5	2.1	R	77
MP18xCML488	2.0	3.0	2.0	2.5	2.4	R	77
A1220-4xA16	**2.0**	**2.5**	**1.5**	**3.5**	2.4	R	80
A16xCML488	3.0	2.5	2.0	2.5	2.5	R	78
N3xA16	2.0	2.5	1.5	5.5	2.9	R	80
A1220-4xCML205	1.0	3.5	2.5	5.0	3.0	R	78
A1220-4xMP18	2.0	3.0	2.5	4.5	3.0	R	78
A1220-4xCZL00001	3.0	2.0	2.5	5.0	3.1	R	77
A1220-4xCML443	2.0	3.0	3.0	5.0	3.3	R	79
N3xMP18	1.5	6.5	1.5	4.5	3.5	R	77
CML445xN3	2.5	3.5	1.5	6.5	3.5	R	78
CZL00001xN3	2.5	3.5	4.0	5.0	3.8	R	76
Checks							
S71	2.5	2.5	1.5	3.0	2.4	R	83
N72	2.5	3.5	2.5	2.5	2.8	R	82
S63	5.0	4.5	4.5	5.5	4.9	MR	75
10 most susceptible hybrids with anthesis between 72 and 82 days							
CZL00001xA16	6.0	7.0	4.0	7.5	6.1	S	76
CML205xCML443	6.0	6.5	4.5	8.0	6.3	S	76
CZL00009xA16	6.5	6.0	5.5	7.5	6.4	S	75
CML445xCZL00001	7.0	5.0	5.5	8.0	6.4	S	77
CML445xCZL00009	8.0	6.5	4.0	8.0	6.6	S	76
CML445xMP18	5.5	7.5	5.5	8.0	6.6	S	78
CML445xA16	8.0	6.0	6.0	6.5	6.6	S	80
CML205xA16	7.0	8.0	4.0	8.0	6.8	S	78
CML445xCML205	7.5	7.0	5.5	8.0	7.0	S	77
CML445xCML443	8.0	6.0	6.5	8.0	7.1	S	78
Checks							
P57	7.0	4.5	3.0	6.0	5.1	MS	77
P067	7.0	7.5	5.5	8.5	7.1	S	79
P77	8.0	8.5	5.5	8.0	7.5	S	75
Mean	4.6	4.9	3.6	5.7	4.7		77
LSD (0.05)	1.8	1.8	1.8	1.9	0.9		1.6
CV (%)	19.5	18.4	24.8	16.3	19.2		1.9

†C108 = Cedara November 2007 planting, C208 = Cedara, January 2008 planting, C09 = Cedara, November 2008 planting, and ZAMB08 = Mpongwe, Zambia, January 2008 planting. ‡Disease reactions: R = resistant, MR = moderately resistant, S = susceptible and MS = moderately susceptible.

Table 4.5 Grain yield (t ha^{-1}) of the 45 F_1 hybrids and nine hybrid checks evaluated over four environments between 2007 and 2009.

ENTRY	CED108†	CED09	ZAMB08	CED208	Mean Yield (t ha-^{1})‡	Anthesis (days)
Hybrids with anthesis between 67 and 74 days						
A1220-4xCZL00009	9.37	10.35	10.40	2.67	8.20	74
CZL00009xN3	8.83	10.16	8.35	3.19	7.63	73
CZL00009xCZL00001	8.63	8.79	8.01	4.24	7.42	73
CZL00009xCML205	7.75	8.42	6.56	2.60	6.33	71
CZL00009xCML488	5.39	9.61	6.56	2.98	6.13	72
CZL00009xMP18	6.46	8.85	5.65	2.36	5.83	72
Checks						
S51	7.29	7.61	8.00	2.02	6.23	72
P17	8.46	7.32	7.46	1.80	6.26	74
P27	11.10	8.88	6.64	2.47	7.27	73
Top yielding 20 hybrids with anthesis between 75 and 81 days						
A1220-4xA16	8.74	11.61	10.22	5.65	9.05	80
CZL00009xA16	9.38	10.03	8.80	6.05	8.56	75
A1220-4xN3	9.49	8.98	10.97	3.18	8.15	79
N3xCML443	8.95	11.12	9.75	2.79	8.15	77
A1220-4xCZL00001	9.15	10.29	8.25	4.79	8.12	77
CZL00001xA16	8.76	10.91	9.44	3.30	8.10	76
N3xA16	9.33	7.76	10.45	4.67	8.05	80
A16xCML443	9.18	11.80	7.72	2.47	7.79	81
CML445xCML443	10.12	9.58	7.56	3.67	7.73	78
CML445xN3	8.96	9.63	9.21	3.04	7.71	78
A1220-4xCML488	8.70	9.87	8.15	3.93	7.66	77
N3xCML488	8.30	8.29	8.39	5.57	7.63	76
CML445xCML488	7.34	11.64	7.81	3.49	7.57	76
CZL00009xCML443	8.68	10.59	7.43	3.40	7.52	75
A16xMP18	8.00	9.43	7.53	4.70	7.41	78
CML445xCZL00009	8.53	10.41	6.56	3.67	7.29	76
A1220-4xCML443	8.34	9.60	7.59	3.50	7.26	79
A16xCML488	7.07	10.75	6.91	4.08	7.20	78
MP18xCML443	8.75	8.91	8.83	2.19	7.17	77
A1220-4xCML205	7.32	10.98	4.97	5.38	7.16	78
Checks						
S63	12.97	10.57	8.36	2.36	8.56	75
P77	7.55	9.41	6.68	3.41	6.76	75
P57	9.41	7.84	7.25	1.66	6.54	77
S71	10.12	9.30	7.97	2.18	7.39	83
P067	9.70	10.53	8.55	3.22	8.00	79
N72	8.55	11.13	8.45	3.84	8.00	82
MEAN	8.25	9.40	7.60	3.38	7.15	77
LSD (0.05)	2.14	2.50	2.23	2.27	1.13	1.62
CV (%)	12.90	13.30	14.60	33.50	16.00	1.86

.†C108 = Cedara November 2007 planting, C208 = Cedara January 2008 planting, C09 = Cedara November 2008 planting, and ZAMB08 = Mpongwe, Zambia January 2008 planting. ‡Mean yield = average yield across the four environments.

Table 4.6 Combined analysis of variance for PLS disease, grain yield (tha^{-1}), days to anthesis, days to silking, plant height (cm), ear height (cm) and relative ear position of 45 F_1 hybrid crosses tested in different environments between 2007 and 2009 and the contribution of the different genetic effects to the total hybrid sum of squares.

	PLS		Grain Yield (t ha^{-1})		Days to 50% anthesis		Days to 50% silking		Plant height (cm)		Ear height (cm)		Relative Ear Position	
Source	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS
Environment (E)	3	77.91***	3	568.25***	2	11214.06***	2	12396.58***	3	232000.97***	3	80840.06***	3	0.043***
Replication(E)†	4	1.99*	4	10.34***	3	4.83^{ns}	3	1.68^{ns}	4	828.49***	4	370.03***	4	0.889^{ns}
Hybrid	44	22.96**	44	5.27***	44	28.54***	44	32.16***	44	714.21***	44	821.72***	44	0.008***
GCA	9	100.98***	9	16.97***	9	122.29***	9	131.15***	9	2742.85***	9	3178.42***	9	0.026***
SCA	35	2.90***	35	2.26**	35	4.44***	35	6.71***	35	192.56	35	215.71***	35	0.002
E*Hybrid	132	2.07***	132	2.46***	88	12.22***	88	11.99***	132	236.22**	132	210.05***	132	0.003***
GCA*E	27	3.98***	27	3.64***	18	29.56***	18	35.33***	27	431.22***	27	351.13***	27	0.003***
SCA*E	105	1.58***	105	2.15***	70	7.76***	70	5.99***	105	186.08^{ns}	105	173.78***	105	0.002**
Error	176	0.83	176	1.37	132	2.11	132	2.25	176	156.29	176	104.54	176	0.002
CV	20.34		16.40		1.85		1.93		5.04		7.97		7.73	
%GCA contribution (SS)	**89.95**		65.80		87.64		83.41		78.55		79.12		74.46	
%SCA contribution (SS)	**10.05**		34.16		12.36		16.59		21.45		20.88		25.54	

*, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001, respectively, ns = non-significant (P>0.05). †This indicates replications were nested within environments.

4.3.3 Combining ability estimates

Mean Squares for environment, hybrid, general combining ability (GCA) effects, specific combining ability (SCA) effects and all the interactions were highly significant (P≤0.001) for PLS diseases (Table 4.6). The GCA effects for PLS disease were 9 times larger than the SCA effects. On partitioning the hybrid sum of squares, the GCA effects accounted for almost 90% and the SCA about 10% of the variation among the hybrid PLS scores.

Mean Squares for environment, hybrid, GCA and SCA effects for grain yield, days to anthesis, days to silking, plant height, ear height and relative ear position were all highly significant (P≤0.01), with the exception of the SCA effects for plant height and relative ear position (Table 4.6). All the interactions with the environment for hybrids, GCA and SCA effects were significant (P≤0.01), except for SCA x environment for plant height. The ratio of the GCA to SCA effects for all these agronomic traits, that is, grain yield, days to 50% anthesis, days to 50% silking, plant height, ear height and relative ear position followed the same trend. The GCA effects were about 1.9 to 7.0 times larger than the SCA effects amongst the various traits. On partitioning the hybrid sum of squares of the different traits, the GCA effects accounted for about 66% to 88% and the SCA effects about 12% to 34% of the variation among the hybrids.

4.3.4 General combining ability estimates of the inbred parents

The GCA effects, mean disease scores and grain yield of the ten parents for the different traits measured are presented in Tables 4.7. The GCA effects for Phaeosphaeria leaf spot (PLS) were highly significant (P≤0.001) in the different environments. The desirable GCA effects for disease resistance should be negative. The GCA effects for parents A1220-4, N3 and CML488 were negative and highly significant (P≤0.01) in all the four environments. Parents; CML445, CZL00009, CML205 and CML443 had positive GCA effects in all the four environments. The other parents; CZL00001 and A16 had positive effects in two environments, whereas for MP18 some of the effects were negative and some positive, depending on the environment.

For yield, desirable GCA effects should be positive. The GCA effects of the ten parents for grain yield, and the other agronomic traits are presented in Table 4.8. Parents with significant (P≤0.05) effects included; A1220-4, N3, CML205, A16, MP18, CML443 and CML488. Positive GCA effects for yield were observed for A1220-4, N3, A16, and CML443 in two of the environments. Parents MP18 and CML488 had negative GCA effects for yield. Overall, across the environments, only A1220-4 and A16 had positive,

significant GCA effects for grain yield, whilst MP18 had negative GCA effects. For days to 50% anthesis and 50% days to silking, negative, significant (P≤0.01) GCA effects were observed for CZL00009, CML205 and CML488 in at least two of the environments. Positive GCA effects for both days to 50% anthesis and silking were recorded for CML445, A1220-4 and A16. Other parental lines such as CZL00001 had negative values for days to anthesis and positive values for days to silking, whilst other lines had some effects positive and some negative depending on the environment. In general across environments, only CZL00009 had significant, negative GCA effects for both days to 50% anthesis and 50% silking.

Parents A1220-4, N3 and A16 had positive, significant (≤0.05) GCA effects for plant height, ear height and ear position. Negative and significant (P≤0.05) GCA effects were observed for; CML488 and CZL00001 (for plant height), CML445, CZL00009, MP18 and CML488 (for ear height) and CZL00009 for relative ear position. Across the environments, only CML488 had negative GCA effects for plant height. Other parents such as A1220-4 and N3 had positive, significant GCA effects for ear height and relative ear position, whereas CZL00009 had negative GCA effects for ear height and ear position across the environments.

Table 4.7 Estimates of general combining ability (GCA)† effects for Phaeosphaeria leaf spot disease scores evaluated in different environments between 2007 and 2009.

Parent	Environment					
	PLS mean score	C108	C09	ZAMB08	C208	Across Environments
CML445	3.8	2.45***	0.76***	0.94***	1.67***	1.45***
A1220-4	4.8	-2.24***	-2.49***	-1.63***	-1.78***	-2.03***
CZL00009	6.0	0.51*	1.08***	0.50*	0.79***	0.72***
CZL00001	5.3	0.31	-0.11	0.88***	0.66***	0.44**
N3	2.3	-1.99***	-1.18***	-1.19***	-0.96***	-1.33***
CML205	5.3	0.70***	1.39***	0.75***	1.16***	1.00***
A16	4.0	0.83***	0.70***	0.19	0.29	0.50***
MP18	3.5	-0.43*	0.89***	0.31	-0.21	0.14
CML443	4.5	1.20***	0.83***	0.63***	1.10***	0.94***
CML488	2.0	-1.36***	-1.86***	-1.38***	-2.71***	-1.83***

†Negative GCA effects were desirable for PLS resistance. *, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001, respectively.

Table 4.8 Estimates of general combining ability (GCA) effects for grain yield (t ha^{-1}), days to anthesis and days to silking, plant height, ear height and relative ear position evaluated in different environments between 2007 and 2009.

Grain Yield (t ha^{-1})							***Days to 50% Anthesis***				***Days to 50% Silking***			
Parent	Mean Grain Yield	C108	C09	ZAMB08	C208	**Across Env†**	C108	C09	ZAMB08	**Across Env**	C108	C09	ZAMB08	**Across Env**
CML445	4.03	-0.26	0.22	0.00	-0.13	**-0.04**	1.53***	1.40**	0.08	**1.00**	1.50***	0.91	-0.11	**0.77**
A1220-4	2.16	0.25	0.84***	0.75***	0.59*	**0.61***	1.28***	1.15**	1.33***	**1.25**	1.44***	1.23**	1.08***	**1.25**
CZL00009	3.98	0.12	0.32	0.01	-0.1	**0.09**	-3.41***	-5.10**	-2.80***	**-3.77****	-4.63***	-4.46***	-2.80***	**-3.96****
CZL00001	3.30	-0.19	0.39	-0.34	0.49	**0.09**	-0.79**	0.03	-0.68**	**-0.48**	1.00***	1.73***	-0.3	**0.81**
N3	5.43	0.85***	-0.74**	1.30***	-0.17	**0.31**	-0.16	-0.23	0.64**	**0.08**	0.19	0.54	1.26***	**0.66**
CML205	1.82	-0.45	-0.44	-1.01***	-0.35	**-0.56**	-1.35***	0.09	-0.99***	**-0.75**	-0.38	0.16	-0.3	**-0.17**
A16	1.74	0.48*	0.38	1.17***	0.80***	**0.71****	1.84***	2.65***	1.33***	**1.94**	1.94***	1.60***	1.14***	**1.56**
MP18	2.03	-0.69***	-1.50***	-0.90***	-0.77**	**-0.97*****	2.84***	-2.66***	0.14	**0.1**	2.19***	-3.34***	0.08	**-0.36**
CML443	3.91	0.54*	0.62*	-0.11	-0.5	**0.14**	-0.60*	3.59***	0.45	**1.15**	-1.44***	3.98***	0.26	**0.93**
CML488	2.3	-0.65**	-0.08	-0.87	0.14	**-0.36**	-1.16***	-0.91*	0.51*	**-0.52**	-1.81***	-2.34***	-0.3	**-1.48**

Plant height (cm)						***Ear height (cm)***					***Relative ear position***				
Parent	C108	C09	ZAMB08	C208	**Across Env**	C108	C09	ZAMB08	C208	**Across Env**	C108	C09	ZAMB08	C208	**Across Env**
CML445	-0.81	-1.38	-1.56	-6.67	**-2.61**	-3.49	1.34	-1.81	-4.98*	**-2.23**	-0.01	0.01	0.00	-0.01	**0.00**
A1220-4	3.25	6.38**	11.56***	7.86*	**7.26**	11.23***	10.09***	12.56***	3.3	**9.30****	0.03***	0.02***	0.03***	0.00	**0.02*****
CZL00009	-2.22	-2.56	-1.56	-5.19	**-2.88**	-14.86***	-19.68***	-4.63	-13.92***	**-13.27****	-0.04***	-0.07***	-0.02*	-0.06***	**-0.05*****
CZL00001	-12.38***	1.63	-2.81	4.66	**-2.23**	-10.86***	-1.71	-3.06	-2.79	**-4.6**	-0.01	-0.01	-0.01	-0.02	**-0.01****
N3	15.91***	6.25**	10.94***	3.48	**9.14**	15.23***	4.39	7.56**	10.41***	**9.40****	0.02**	0.00	0.01	0.04***	**0.02*****
CML205	-0.34	-0.75	-4.38	-1.95	**-1.86**	4.15	0.56	-2.75	-1.46	**0.12**	0.01	0.00	0.00	0.00	**0.00**
A16	13.72***	5.00*	8.75***	7.67*	**8.79**	4.11	4.47	7.25**	10.18***	**6.5**	-0.01	0.01	0.01	0.03**	**0.01**
MP18	1.84	-3.44	-4.69	5.31	**-0.24**	0.36	-4.43	-5.25*	3.07	**-1.56**	0.00	-0.01	-0.01	0.00	**-0.01**
CML443	-1.75	5.94**	-9.69***	-9.67**	**-3.79**	0.8	11.66***	-4.31	-3.29	**1.21**	0.00	0.03***	0.01	0.01	**0.01****
CML488	-17.22***	-17.06***	-6.56**	-5.5	**-11.59***	-6.67**	-6.71**	-5.56*	-0.53	**-4.87**	0.01	0.01	-0.01	0.01	**0.00**

†Env = environments. *, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001, respectively.

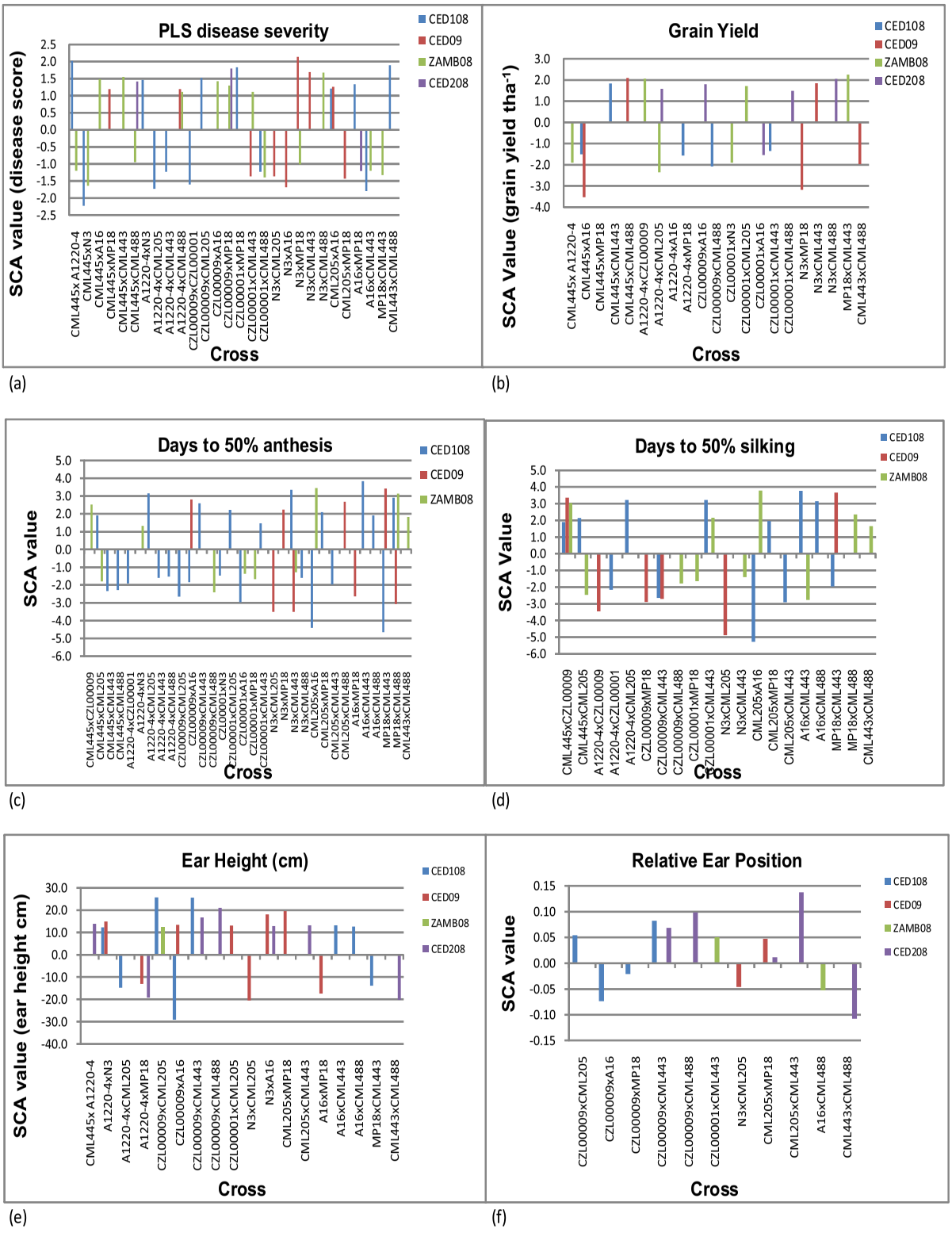

Figure 4.1 . Positive and negative significant mean estimates of specific combining ability (SCA) effects for the set of diallel crosses among ten maize inbred lines in different environments.

4.3.5 Specific combining ability estimates for PLS disease

Specific combining ability estimates for PLS diseases in the different environments are shown in Fig 4.2 and the combined effects across environments in Table 4.9. The SCA effects were variable in the different environments. The C208 environment had only one hybrid with negative significant (P≤0.05) SCA effects. For the other three environments; C108, C09 and ZAMB08, the number of hybrids with significant negative SCA effects ranged between four and seven. Most of these hybrids involved resistant parents only but a few were between a susceptible and resistant parent. Three hybrids; CML445 x N3, CZL00001 x CML488 and A16 x CML443 had significant (P≤0.05) negative SCA effects in at least two environments, whereas the other hybrids had significant (P≤0.05) negative SCA effects in at least one environment. The hybrids CML445 x A1220-4, N3 x MP18 and CML443 x CML488 had the highest positive, significant (P≤0.01) SCA effects. Overall, only CML445 x N3, A1220-4 x CZL00009 and CZL00009 x CML488 had significant negative SCA effects across the environments (Table 4.9).

4.3.6 Specific combining ability effects for grain yield and the other agronomic traits

Significant SCA effects for grain yield and other agronomic traits are presented in Fig 4.2b-d. The SCA effects were highly variable in the different environments. Ten hybrids had significant (P≤0.05) positive SCA effects for grain yield in the different environments. About 20 hybrids showed negative SCA effects for 50% to anthesis and 13 hybrids for 50% to silking in the three environments. Nineteen hybrids had positive SCA effects for 50% to anthesis and about 12 hybrids for 50% to silking. Hybrids involving CML445, A1220-4, CZL00009, CZL00001 and MP18 had mostly negative SCA effects for anthesis and silking.

The SCA effects and the SCA x environment for plant height were not significant (Table 4.6), so they are not presented. Specific combining ability effects for ear height and relative ear position are presented in Figs 4.2e-f. The hybrids reacted differently for ear height and relative ear position in the different environments. However, all the SCA effects for grain yield, 50% to anthesis, days to 50% silking, ear height and relative ear position were not significant across the environments (data not shown).

Table 4.9 Combined estimates of specific combining ability (SCA) effects for PLS disease scores and estimates of percentage mid-parent and better-parent heterosis for PLS disease and grain yield (tha^{-1}), for the 45 F_1 hybrids evaluated between 2007 and 2009.

Cross	PLS Mean Score	Cross Type	PLS SCA effects†	PLS Heterosis†		Grain Yield Heterosis‡	
				%MPH	%BPH	%MPH	%BPH
CML445x A1220-4	4.0	R x MR	0.09	11.8	26.7	189.5	122.2
CML445xCZL00009	6.6	R x S	-0.03	48.7	93.3	136.5	135.2
CML445xCZL00001	6.4	R x MS	0.00	33.3	60.0	136.5	115.2
CML445xN3	3.5	R x R	-1.11**	0.0	30.4	96.7	71.3
CML445xCML205	7.0	R x MS	0.06	61.1	93.3	166.1	93.0
CML445xA16	6.6	R x R	0.19	80.6	86.7	128.5	63.7
CML445xMP18	6.6	R x R	0.55	79.3	85.7	166.9	100.8
CML445xCML443	7.1	R x MR	0.25	69.7	84.2	148.4	144.7
CML445xCML488	4.1	R x R	0.01	47.8	112.5	200.1	135.7
A1220-4xCZL00009	2.3	MR x S	-0.92*	-62.8	-57.9	221.2	147.5
A1220-4xCZL00001	3.1	MR x MS	0.23	-50.0	-47.4	256.3	194.5
A1220-4xN3	1.6	MR x R	0.50	-64.3	-44.4	143.6	70.2
A1220-4xCML205	3.0	MR x MS	-0.45	-55.0	-52.6	360.7	324.4
A1220-4xA16	2.4	MR x R	-0.58	-48.6	-43.8	422.0	372.0
A1220-4xMP18	3.0	MR x R	0.40	-39.4	-28.6	251.4	241.4
A1220-4xCML443	3.3	R x MR	-0.14	-45.9	-44.4	196.0	129.6
A1220-4xCML488	1.5	MR x R	0.87	-63.0	-37.5	316.8	303.9
CZL00009xCZL00001	5.5	S x MS	-0.14	-15.6	-9.5	139.1	118.6
CZL00009xN3	4.0	S x R	0.12	-3.0	77.8	101.8	74.9
CZL00009xCML205	6.8	S x MS	0.55	33.3	42.9	178.9	103.0
CZL00009xA16	6.4	S x R	0.67	25.0	56.3	239.0	143.6
CZL00009xMP18	5.9	S x R	0.53	5.3	42.9	154.5	92.2
CZL00009xCML443	6.0	S x MR	-0.14	23.8	44.4	144.2	141.8
CZL00009xCML488	2.8	S x R	-0.63*	-18.8	62.5	138.8	88.3
CZL00001xN3	3.8	MS x R	0.15	-20.0	33.3	95.9	57.6
CZL00001xCML205	5.9	MS x MS	-0.05	9.5	9.5	232.4	157.6
CZL00001xA16	6.1	MS x R	0.70	40.5	62.5	290.0	198.0
CZL00001xMP18	5.0	MS x R	-0.06	20.0	50.0	194.1	137.7
CZL00001xCML443	6.0	MS x MR	0.14	2.6	11.1	143.4	124.5
CZL00001xCML488	2.1	MS x R	-0.97	-37.9	12.5	205.0	158.7
N3xCML205	3.9	R x MS	-0.28	-13.3	44.4	154.2	69.6
N3xA16	2.9	R x R	-0.78	-28.0	0.0	138.4	57.5
N3xMP18	3.5	R x R	0.20	39.1	77.8	64.1	12.8
N3xCML443	4.8	R x MR	0.65	55.6	133.3	115.1	85.0
N3xCML488	1.9	R x R	0.55*	-29.4	-25.0	114.8	52.9
CML205xA16	6.8	MS x R	0.76	62.2	87.5	396.0	386.1
CML205xMP18	4.9	MS x R	-0.75	2.9	28.6	272.7	252.8
CML205xCML443	6.3	MS x MR	-0.17	28.2	38.9	195.0	116.1
CML205xCML488	4.0	MS x R	0.34	17.2	112.5	270.9	232.0
A16xMP18	5.5	R x R	0.37	73.3	85.7	361.7	328.8
A16xCML443	5.3	R x MR	-0.67	23.5	31.3	271.5	168.6
A16xCML488	2.5	R x R	-0.66	-8.3	37.5	341.0	287.7
MP18xCML443	4.8	R x MR	-0.81	31.3	50.0	197.3	126.1
MP18xCML488	2.4	R x R	-0.42	-9.1	25.0	259.8	239.1
CML443xCML488	4.5	MR x R	0.90*	61.5	162.5	157.4	104.4
Mean				9.34	37.9	202.0	151.9
LSD (0.05)				8.6	8.0	12.3	9.8

*, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001, respectively. †Negative SCA effects and heterosis estimates were desirable for PLS resistance, while ‡positive heterosis estimates were desirable for grain yield.

4.3.7 Estimates of heterosis

Mid-parent heterosis (MPH) and better-parent heterosis (BPH) estimates for PLS and grain yield are presented in Table 4.9. Both the MPH and BPH estimates were variable and significant (P<0.001) among the hybrids. Most of the hybrids had MPH of more than 100%. For grain yield, the MPH and BPH were all positive and ranged from 64 to 422% and 13 to 372%, respectively. The highest amount of heterosis was observed in hybrids involving parents A1220-4 and A16. For PLS disease severity, there were significant (P<0.001) differences among the entries for MPH and BPH. The MPH for PLS ranged from 3 to 64%, while BPH was from 9 to 58%. Hybrids which exhibited negative MPH for PLS were generally the same hybrids that showed a negative BPH, especially for parent A1220-4. These same hybrids were crosses between a resistant and susceptible parent.

4.3.8 Phenotypic correlations

Correlation coefficients amongst the GCA effects and SCA effects of the different traits are presented in Table 4.10. Positive significant correlations (P≤0.01) for GCA effects were detected for anthesis with; silking, ear height and ear position. Significant, positive correlations for GCA effects were also observed between silking with ear height and ear position. The correlations between plant height and ear height and between ear height and ear position were positive.

A negative significant correlation (P≤0.05) for SCA effects was detected between PLS and anthesis (r = -0.31). For anthesis, there was a significant positive correlation for SCA effects with silking. Ear height was positively correlated with plant height and ear position.

The correlations among disease scores for PLS with environments are presented in Table 4.11. There were significant (P≤0.001) and positive correlations for PLS scores with all the environments (r ≈ 0.70).

Table 4.10 Pearson correlation coefficients among SCA effects (above diagonal) for 45 F_1 hybrids and GCA effects (below diagonal) for 10 inbred parent lines for the three diseases, grain yield and five secondary traits in the 45 F_1 hybrids evaluated in different environments

	PLS	Grain Yield (t ha^{-1})	Anthesis	Silking	Plant height	Ear height	Ear position
PLS		-0.13	-0.31*	-0.05	-0.10	-0.22	-0.24
Grain Yield (t ha^{-1})	-0.20		-0.23	-0.19	0.20	0.15	0.11
Anthesis	-0.10	0.33		0.63***	0.18	0.27	0.15
Silking	-0.03	0.37	0.92***		0.11	-0.07	-0.26
Plant height	-0.18	0.60*	0.42	0.52		0.64***	0.13
Ear height	-0.41	0.46	0.78***	0.80***	0.76**		0.83***
Ear position	-0.44	0.26	0.83***	0.80***	0.41	0.90***	

*, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001, respectively.

Table 4.11.Correlations of PLS scores among environments which had significant differences.

	Environment			
	C108	C09	ZAMB08	C208
C108	1.00	0.71***	0.71***	0.72***
C09		1.00	0.69**	0.78***
ZAMB08			1.00	0.76***
C208				1.00

*, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001, respectively.

4.4 Discussion

4.4.1 Disease development

Although the environments appeared different, all the Cedara plantings had high PLS disease pressure and provided a good genetic discrimination for PLS disease among the inbred parents and their hybrids. Correlation coefficients of PLS scores with environments showed a positive correlation for all the four environments; C108, C09, ZAMB08 and C208. This implied that evaluation of PLS in any of these environments would be sufficient for selection of resistant germplasm. This result is in agreement with the

observation made by Vivek *et al*. (2009) for grey leaf spot (GLS) disease scores in different environments and Mawere *et al*. (2006) for maize streak virus (MSV). Therefore, evaluation of the inbreds and hybrids at one reliable site would reduce costs for many of the breeding programmes.

4.4.2 Gene action and combining ability effects

The significant hybrid main effects and interactions for PLS indicated that the hybrids were different and the environments diverse. This interaction could have resulted mainly from differences observed in disease levels in the various locations. The significant GCA and SCA effects observed showed that both additive and non-additive gene effects were important in the resistance to PLS. The GCA effects contributed 90%, whilst the SCA effects accounted for only 10% of the hybrid sum of squares. This indicated that additive gene action was more predominant than non-additive gene action in these inbred lines. These results confirmed that resistance to PLS was predominantly additive as reported by other investigators (Carson, 2001; Silva and Moro, 2004; Mhembere, 2005; Derera *et al*., 2007; Vivek *et al*., 2009). Carson (2001) reported significant but less important dominance effects and no epistasis in the cross B73 x Mo17. Vivek *et al*. (2009) also reported both significant GCA and SCA effects, with a contribution of 65% and 35%, respectively, to the total genetic variation. Derera *et al*. (2007) reported highly significant GCA effects (90% contribution to the total genetic variation) for resistance to PLS suggesting predominance of additive gene action in the crosses evaluated. These significant additive genetic effects imply that selection for increased PLS resistance should be effective and the prediction of crosses to obtain progenies with PLS resistance for these lines could be made based on the GCA effects. It means, therefore, that PLS resistance could be incorporated from resistant sources by utilizing methods such as backcross or recurrent selection, both of which take advantage of additive gene action (Sleper and Poehlman, 2006).

4.4.3 Reaction of inbred parents to PLS infection and combining ability effects

The most resistant (R) parents to PLS included: N3, A16, MP18 and CML488. Of these four lines, only N3 and CML488 had significant, negative GCA effects, which were desirable for PLS resistance. The most susceptible (S) parents to PLS were CZL00009, CZL00001 and CML205. This was consistent with the CIMMYT classification of CZL00009 and CZL00001 as susceptible lines. The PLS scores for these three susceptible lines ranged from moderately susceptible (MS) to susceptible (S), depending on the environment and in addition, they had positive GCA effects. Parental line CML445

had a score of 3.8, which was resistant based on the classification used in this study. However, the line had a positive GCA effect, implying that it would contribute towards susceptibility in the hybrids it was involved. The line had been selected based on being PLS susceptible as its average scores were around 6.0 at Rattray Arnold Research Station (RARS) in Zimbabwe during the 2004/5 season (Derera *et al*., 2007). Derera *et al*. (2007) also observed a positive GCA effect for CML445 when it was used as the female line, and it contributed towards susceptibility in most of the hybrids it was involved. In another study reported in this thesis (Chapter 5), the ratings of CML445 ranged from 3.9-5.8. It appears, therefore, that CML445, although it was resistant in this current study, might be classified as moderately resistant (MR) or moderately susceptible (MS) to PLS. Inbred parent A1220-4 had an overall score of 4.8 (MR), and it had a negative GCA effect, implying that it contributed towards resistance in most of the hybrids it was used. On the other hand, CML443 was also MR (score 4.5), but it had a positive GCA effect and non-significant SCA effects for disease resistance in the hybrids it was involved. In general, some resistant hybrids involved a susceptible and a resistant parent, whereby at least one of the parents had a negative GCA effect. This further confirmed the presence of non-additive gene action.

4.4.4 Reaction of hybrids to PLS infection and combining ability effects

There was significant variation amongst the hybrids for PLS disease resistance. The commercial hybrid checks were amongst the most susceptible to PLS. Although disease pressure for PLS at Cedara for all the three plantings (C108, C09 and C208) was relatively high, about 80% of the hybrids in the late planting (C208) had scores between 4.5 and 8.0, compared to 46 to 53% of the hybrids with the same scores in C108 and C09 environments. In ZAMB08, about 70% of the hybrids had a score of less than or equal to 4.0. The results also showed that there was more disease in the late planted crop at Cedara (C208) in this study and this supported the observation made in Brazil that severe infestations occurred, especially in late plantings (Fernandes, 1998; Cervelatt *et al*., 2002). Increase in PLS disease incidence has been attributed mainly to practices such as late planting, absence of rotation, and zero tillage practices (Casela, 1998; Cervelatti *et al*., 2002). Most of the fields at Cedara were under reduced tillage suggesting high inoculum levels in the plant debris and soil, which could have contributed to the high disease levels in the early plantings (C108 and C09). In Mpongwe (ZAMB08), the distribution of PLS scores was skewed towards resistance. At this site, deep ploughing is practiced, which could lead to a reduction in inoculum at the beginning of the season, and this could explain the relatively low scores observed for most of the hybrids. However,

correlation coefficients of PLS scores with the ZAMB08 environment was positive ($r \approx 0.7$, $P \leq 0.01$). This implied that evaluation of PLS in this environment would provide adequate genetic discrimination for both the inbred parents and hybrids and allow selection of resistant germplasm.

The hybrids with negative, significant SCA effects involved the resistant parents A16, CML488, MP18 and N3 in the different environments. However, the MR parent A1220-4 (score 4.8), which had a negative GCA effect contributed towards resistance in most of the hybrids it was involved in the various environments. Across the environments, only three hybrids CML445 (MR) x N3 (R), A1220-4 (MR) x CZL00009 (S) and CZL00009 (S) x CML488 (R) had significant negative SCA effects. These crosses involved parents with differences in resistance levels and some between a resistant and a susceptible parent. The results, therefore, showed that for PLS, susceptible parents could be used in combination with resistant parents to produce resistant hybrids. Therefore, the significant SCA effects that were observed towards reduced disease imply that non-additive gene effects can be utilized in hybrid development.

4.4.5 Combining ability effects for the other agronomic traits

The environment, hybrid, GCA and SCA effects for grain yield, days to anthesis, days to silking, plant height, ear height and relative ear position were all highly significant, with the exception of the SCA effects for plant height and relative ear position. This indicated that both additive and non-additive gene action were important for grain yield, days to anthesis, days to silking and ear height, whereas for plant height and ear position, only additive gene-action was important. The significant interactions with the environment for the hybrids, GCA and SCA effects imply that the inbreds or hybrids in one environment may behave differently for the same trait in a different environment. This could influence the breeding strategy towards breeding for specific adaptation. The GCA effects were higher than the SCA effects, indicating that additive gene action was more predominant than the non-additive component. Therefore, high grain yield could be improved using methods such as pedigree breeding, single seed descent or early generation selection (Moreno-Gonzalez and Cubero, 1993).

The late planting (C208) at Cedara had lower yields than the early planted crop (C108 and C09) and ZAMB08. The late planting in C208 was affected by the seasonal rainfall distribution. A mid-season drought was experienced in this environment from around the mid-January 2008 to the end of March 2008 and no supplementary irrigation was applied.

The rainfall was very erratic with only a total of 131.4 mm being recorded during that period. In addition, the long maturing varieties could have been disadvantaged by the short season, thus resulting in lower yields. The hybrids in general were high yielding in C108, C09 and ZAMB08 environments, but low yielding in C208. This showed that most of the hybrids were probably adapted to high potential environments and belonged to the medium to late maturing groups. The C208 environment was conducive for selection of hybrids that perform well under drought-stress environments or short-season varieties.

Parents A1220-4, N3, CML205, A16, and CML443 all had positive significant GCA effects for grain yield and they contributed towards higher yields in some of the hybrids they were involved in various environments. However, overall across environments, only A1220-4 and A16 had positive, significant GCA effects for grain yield whilst MP18 had negative GCA effects. For days to 50% anthesis and silking, negative, significant GCA effects indicated early maturity and these were observed for CZL00009 across the environments. However, other parents such as CML205 and CML488 also had negative SCA effects in some of the environments, but not across the environments. Positive GCA values for both days to anthesis and silking indicated late maturity and were recorded for CML445, A1220-4 and A16. Other parental lines such as CZL00001 had negative values for days to anthesis and positive values for days to silking, which could imply a large anthesis-silking interval (ASI). A large ASI could result in lack of synchronization of the pollen and silks in self pollinations. This could be a potential problem in hybrid production as it has a direct bearing in the maintainance of the inbred lines. The problem, however, is often resolved through staggered planting. In general, the results indicated that parents A1220-4 and A16 had good combining ability for grain yield but were late in maturity as depicted by the GCA effects for days to anthesis and silking. It is possible, however, to take advantage of the high grain yield and select these lines for high potential environments, with uniform rainfall distribution or supplementary irrigation. The parent CZL00009 was the most promising line for early maturity.

Significant SCA effects for grain yield, 50% to anthesis and days to 50% silking were highly variable in the different environments. Overall, none of the hybrids had significant SCA effects for any of these three traits (data not shown). This showed that breeding for specific adaptation was an important factor for grain yield and flowering in these hybrids. In addition, a small negative, but significant ($P \leq 0.05$) correlation for SCA effects was observed between PLS scores with anthesis ($r = -0.31$). This implied that, the earlier the hybrid in flowering, the more severe was the PLS disease. Parents A1220-4, N3 and A16 had positive, significant (≤0.05) GCA effects for plant height, ear height and relative ear

position in at least three of the environments. This is an indication of taller plants and higher ear placement. These same three parents had high positive GCA effects for grain yield and contributed towards late maturity. The height components were positively correlated with the flowering components for both GCA and SCA effects. This showed that the late the hybrids in maturing, the taller were the plants and the higher was the ear placement.

Plant height and ear placement are important factors in development of cultivars that are resistant to lodging. In most cases shorter plants with strong root systems are preferred (Sleper and Poehlman, 2006). Negative significant GCA effects were observed for CML488 and CZL00001 for plant height, for CML445, CZL00009, MP18 and CML488 for ear height and CZL00009 for relative ear position. This indicated that these parents contributed towards shorter plants and lower ear placement. In general, across the environments only CML488 had negative GCA effects for plant height. The SCA effects and the SCA x environments for plant height were not significant. This showed uniformity in plant heights among the hybrids in the different environments.

4.4.6 Estimates of heterosis

Mid-parent and better-parent heterosis was observed for PLS disease and grain yield. Heterosis confirmed the importance of non-additive gene action for these traits. Although it is generally accepted that heterosis to a large extent, is due to dominance gene action, however, both epistasis and over-dominance also are important (Singh, 1993). Falconer and Mackay (1996) indicated that the amount of heterosis was specific to each cross. Positive heterosis for grain yield was desirable and generally, hybrids involving parental lines A1220-4, CML205, A16 and CZL00001 had higher mid-parent and better-parent heterosis estimates. According to Sleper and Poehlman (2006), for a hybrid plant to be useful to the farmer or breeder, it had to exceed the best parent in yield and productivity. Negative mid-parent heterosis and better-parent heterosis for the diseases further confirmed the significance of non-additive effects in hybrid production. For PLS disease, mostly crosses involving the moderately resistant (MR) parent (A1220-4), CZL00009, susceptible (S), CZL00001, moderately susceptible (MS) and N3 resistant (R) resulted in hybrids showing negative heterosis. These results also confirm observations by Derera (2005) who reported relatively high mid-parent heterosis values for the R x S and MR x S crosses, ranging from 4 to 53% towards PLS resistance.

4.5 Conclusion

The inbred lines that were resistant to PLS observed in this study included A1220-4, N3, A16, MP18 and CML488. The lines A1220-4, N3 and CML488 had negative GCA effects indicating good combining ability for PLS resistance and contributed negative SCA effects in most of the crosses, in the various environments. Across environments about 51% of the hybrids showed high levels of PLS resistance. Parental lines A1220-4 and A16 contributed towards high grain yield and they were late in maturity. The line CZL00009 was early maturing. The hybrids with significant negative SCA effects for PLS were CML445 (MR) x N3 (R), A1220-4 (MR) x CZL00009 (S) and CZL00009 (S) x CML488 (R). Hybrid A1220-4 (MR) x CZL00009 (S) had the highest negative MPH and BPH and line A1220-4 (negative GCA effects) contributed towards high negative heterosis in most of the hybrids it was involved.

General combining ability (GCA) accounted for 90% of the hybrid sum of squares for PLS, whilst SCA effects contributed only 10%. GCA effects for grain yield and the other agronomic traits accounted for 65 to 87% of the hybrid sum of squares. This indicated the predominance of genes with additive over non-additive gene effects for PLS resistance, grain yield and the other agronomic traits in these inbred lines. Overall, highly significant additive effects imply that progress in PLS disease resistance and high grain yield would be made through methods such as pedigree breeding or early generation selection. The results also showed that it is possible to use PLS susceptible parents crossed with resistant parents to produce resistant hybrids. Therefore, the significant SCA effects that were observed towards reduced disease and high yield imply that non-additive gene effects can be utilized in hybrid development.

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5 Generation Mean Analysis of Phaeosphaeria Leaf Spot Resistance in Six Tropical Advanced Maize Inbred Lines

Abstract

Phaeosphaeria leaf spot (PLS) disease, caused by *Phaeosphaeria maydis* (Henn.), has been increasing in severity across eastern and southern Africa since the early 1990s and has great potential of causing yield losses ≥60% in susceptible maize varieties. However, PLS resistance that is available in the important tropical inbreds that dominate hybrid parentage in tropical Africa has not been studied. Knowledge of inheritance or the nature of gene effects controlling PLS resistance in these inbred lines would be useful in designing single cross hybrids and breeding new lines with enhanced resistance. This study was conducted to determine the inheritance of PLS resistance in populations involving six tropical advanced maize inbred lines. Reciprocal crosses and backcross progenies were generated among A1220-4, A15, B17 (resistant, R), CML445 (moderate, MR), CML441 and CZL00001 (susceptible, S) lines. These were evaluated for PLS resistance in two replications for two seasons at Cedara Research Station in South Africa. A 10 parameter model for the data was subjected to generation mean analysis in SAS. Results indicated highly significant additive effects ($P<0.001$) in controlling PLS resistance, while dominance effects accounted for ≤11% of the variation. There was evidence for significant role of epistasis, and of cytoplasmic gene effects which were in favour of resistance in F_1 crosses when the more susceptible parent was used as female. Transgressive segregation for both resistance and susceptibility was also observed. Resistance was medium to highly heritable and conditioned by less than four genes which exhibited incomplete dominance. Dominance and cytoplasmic gene effects which were associated with reduced disease levels can be exploited in hybrid production. Nonetheless, selection would be effective to improve resistance, but observation of epistasis and significant generation x season interaction effects might present challenges.

5.1 Introduction

Among other biotic constraints, Phaeosphaeria leaf spot (PLS) disease threatens food security in sub-Saharan Africa (SSA), if susceptible varieties are grown (Derera *et al*., 2007). The disease caused by the ascomycete fungus *Phaeosphaeria maydis* (Henn.) Rane, Payak & Renfro was first reported from India (Rane *et al*., 1965) but is now widely distributed. It has been reported mainly from Central and South America, India, Central, East and Southern Africa, the United States of America (USA) and Hawaii (De Leon, 1984). Over the past years, PLS disease has been steadily building up resulting in significant damage on maize. In Brazil, for example, incidence and severity of PLS has increased from the mid 1980s causing severe grain yield reductions of more than 60% in susceptible maize cultivars (Casela, 1998; Paccola-Meirelles *et al*., 2001). Carson (2005) in a study conducted in the USA also reported a reduction in grain yield of 11 to 13%. In southern and eastern Africa, PLS disease incidence has been on the rise since the early 1990s (Mwangi, 1998; Smit and Lawrence, 2004; Mhembere, 2005; Vivek *et al*., 2009), resulting in great concern from farmers, maize breeders and pathologists, given the potential it has of causing yield losses. Therefore research on the improvement of PLS resistance in maize is becoming increasingly important in most breeding programmes.

Few studies have identified resistance sources mostly on American and Brazilian lines. Work done in Brazil and India showed significant differences in resistance of inbreds and open-pollinated varieties (OPVs) to PLS disease (Das *et al*., 1989b; Pegoraro *et al*., 2002, Silva and Moro, 2004). In the USA, evaluations of the reaction of numerous temperate germplasm, indicated that inbred lines derived from Mo17 are highly resistant, whereas those derived from B73 are particularly susceptible to PLS disease (Carson, 2001). However, maize germplasm that performs well in temperate regions generally cannot be introduced into non-temperate regions without undergoing extensive local adaptation (Morris, 2002). Therefore, most of the improved varieties grown in the United States are of little direct use in tropical environments.

Development of disease resistant genotypes depends upon an understanding of the genetic variability and inheritance of the resistance for effective selection to be conducted. In studies from India, Das *et al*. (1989a) reported resistance to PLS to be dominant. However, other studies on American maize lines have indicated that the resistance is inherited quantitatively and is incompletely dominant (Carson, 2001). Estimates of the number of genes involved in the inheritance of resistance ranged from three to four (Carson, 2001). Pegoraro *et al*. (2002) using Brazilian lines observed two

major independent genes that were involved in the inheritance of resistance to PLS disease.

In addition, additive gene action was shown to be more important for PLS disease inheritance than dominant gene action in the American and Brazilian lines (Carson, 2001; Silva and Moro, 2004; Mhembere, 2005; Derera *et al*., 2007; Vivek *et al*., 2009). Carson (2001) reported that dominance genetic effects were significant in the cross B73 x Mo17, but accounted for less than 10% of the variation in PLS resistance among generation means. However, in that same study using B73 x Mo17, there were no epistatic effects or transgressive segregation observed (Carson, 2001). On the other hand, general combining ability (GCA) contributed 65% and specific combining ability (SCA) 35% of the variation in PLS resistance in studies conducted by Vivek *et al*. (2009) in African maize. Derera *et al*. (2007) reported a 90% contribution for the GCA component in southern African maize, indicating predominantly additive gene action. In contrast, Das *et al*. (1989b) using a diallel cross of eight open pollinated varieties of maize found significantly higher levels of dominance variance than additive effects on the genetic control of PLS disease resistance in Indian maize. Derera *et al*. (2007) also reported the predominance of the female GCA over the male GCA in the southern African germplasm. This suggested the influence of cytoplasmic inheritance and warrants further investigations. The presence of cytoplasmic inheritance influences the choice of the female parents to be used in single crosses if the levels of resistance are to be enhanced.

It would be important to study the PLS resistance which is available in the important tropical inbreds that dominate hybrid parentage in tropical Africa as information is still limited. Knowledge generated on the inheritance or the nature of gene effects controlling PLS resistance in these inbred lines would aid in designing single cross hybrids and breeding new lines with enhanced resistance. The lines under study were derived from lines such as; CML395, CML444, CML206, SC and N3, which form the basis of most productive hybrids in medium to high altitude mega-environments in tropical east and southern Africa. Some were from ZM605 which is a highly productive synthetic population for mid-altitude environments in Africa. This study was therefore conducted to: i) determine the inheritance of PLS resistance from the six tropical advanced inbreds (that is, beyond S_6 generation) using generation mean analysis, ii) investigate the existence of cytoplasmic inheritance of PLS, iii) estimate heritability and the number of genes contributing to PLS resistance, and iv) determine heterosis and average degree of dominance on PLS resistance in African maize lines.

5.2 Materials and methods

5.2.1 Crosses for generation mean analysis

The F_1 single crosses were generated from six tropical elite inbred lines obtained from CIMMYT programme in Harare, Zimbabwe. The inbred parents used for the crosses are indicated in Table 5.1. These lines were sampled from the heterotic groups that are a significant source of inbred lines used in hybrid production in east and southern Africa. The lines were further selected based on their maturity dates and reactions to PLS. The single crosses included three resistant (R_1, R_2 and R_3), one moderately resistant (MR) and two susceptible (S_1 and S_2) parents from different genetic backgrounds and crossed as follows: R_1 x S_1; R_2 x S_1; MR x S_1; MR x S_2; R_1 x R_2, R_1 x R_3; R_2 x R_3 and S_1 x S_2. These different classifications will be referred to as a cross in this study. The other crosses made from the F_1 single cross hybrid will be referred to as generations.

The resulting F_1 single cross hybrids and their reciprocals (F'_1) were selfed and backcrossed to each of the parents to produce F_2 , F'_2 and $BCP_1(F_{1)}$, $BCP_1(F'_1)$ and BCP_2 (F_1) and $BCP_2(F'_1)$ generations, where P_1 was the first parent and P_2 the second parent in each cross. These generations were produced during winter of 2007 at the University of KwaZulu-Natal (UKZN), Pietermaritzburg, South Africa (SA).

Table 5.1 Parental lines used for generation mean analysis

Designation†	Pedigree	Heterotic‡ grouping	Principal Selection criteria
A1220-4	[(CML395/CML444)-B-4-1-3-1-B/CML395//SC/ZM605#b-19-2-X]-1-2-X-1-1-BBBBBB]-7-1-3-2-BBB	B / SC	PLS resistant
A15	[CML197/N3//CML206]-X-32-1-4-B-B-B-B	A / N3	PLS resistant
B17	[LZ956441/LZ966205]-B-3-4-4-B-5-B-B-B-B	B	PLS resistant
CML441	ZM605C2F1-17-1-B-1-BB	B	PLS susceptible
CML445	[[TUXPSEQ]C1F2/P49-SR]F2-45-7-5-1-BBB	AB	PLS moderately resistant
CZL00001	INTA-191-2-1-2-BBBB	A	PLS susceptible

†A1220-4, A15 and B17 were coded for convenience of study. ‡Group A and B are Mo17 and B73 oriented, respectively.

5.2.2 Field Evaluation of the different generations

The different generations were evaluated in 2007/8 and 2008/9 seasons at Cedara Agricultural Research Station (29°31'S and 30°16'E, 1130 m altitude) in South Africa. Trials were hand-planted on 8 January 2008 for the 2007/8 season and 27 November

2008 for the 2008/9 season. Each cross was considered to be a separate experiment, but the general procedures applied were the same for all the crosses. The experiment was laid out as a randomized complete block design with two replications. For each cross, there were two rows of each of the P_1, P_2, F_1 and F'_1 generations, eight rows each of the F_2 and F'_2 generations, and four rows of each of the BCP_1 and BCP_2 and their reciprocal generations. Rows were 3.8 m long and 0.75 m inter-row spacing and 0.3 m intra-row spacing in 2007/8 season and 2.8 m long with 0.75 m inter-row spacing and 0.2 m intra-row spacing in 2008/9 season. Plots in 2007/8 season were thinned to 20 plants per row and in 2008/9 season to 15 plants per row. The final number of plants was variable for each generation and is indicated in Table 5.3. A susceptible inbred line was used as a spreader and planted at the borders of each block. Fertiliser was applied at the rate of 120kg N, 33 kg P, 44 kg K. Standard cultural practices including ploughing and disking, hand planting, hand weeding and/or application of herbicides and fertilizers were practiced each season. Disease severity was rated by visually estimating the percent leaf area blighted (necrotic) on individual plants following a 1-9 logarithmic increment rating scale (Table 5.2) at the hard dough developmental stage of maize. Three crosses were rated per day due to the large numbers of plants involved.

Table 5.2 Disease Scale used for rating PLS disease

Score	Description	% Leaf area showing disease symptoms	Disease Reaction
1	no visible symptoms	0	Symptomless
2	A few lesions scattered on lower leaves	<1	Very highly resistant
3	Few scattered lesions, mostly on lower leaves only, but not linked together.	1-3	Highly resistant
4	Moderate number of lesions.	4-6	Resistant
5	Abundant lesions on lower leaves and a few on middle leaves, a few portions of the leaf necrotic.	7-12	Moderately resistant
6	Abundant lesions, some linked together to form necrotic (dead) areas.	13-25	Moderately susceptible
7	Necrotic areas linked together and a few tips dead, lower, middle leaves all showing symptoms, extending to upper leaves.	26-50	Susceptible
8	25% of the leaf tips dead abundant lesions on almost all the leaves, lower leaves dead, more portions of leaf completely blighted.	51-75	Highly susceptible
9	More than 75% of the leaf area diseased or completely blighted, lesions mature and showing black fungal resting structures for both lower and upper leaves. Most of the leaves dead and plant is usually dead.	75-100	Very highly susceptible

Table 5.3 : Number of individual plant ratings used in the calculation of generation means from the eight crosses evaluated for PLS severity at Cedara in 2007/8 and 2008/9 seasons

Cross	Year	Generations and Number of individual plant ratings†									
		P_1	P_2	F_1	F'_1	F_2	F'_2	BCP_1xF_1	$BCP_1xF'_1$	BCP_2xF_1	$BCP_2xF'_1$
A1220-4 (R)xCML441(S)	2007/8	38	40	32	58	104	130	51	43	55	68
	2008/9	42	29	57	55	215	193	72	98	115	91
A15 (R) x CML441 (S)	2007/8	23	17	46	62	173	173	106	93	92	115
	2008/9	47	32	53	53	183	172	106	99	100	88
CML445 (MR) x CML441 (S)	2007/8	29	24	47	35	188	239	85	85	95	68
	2008/9	41	38	42	50	182	199	70	93	42	106
CML445(MR) x CZL00001 (S)	2007/8	33	31	42	37	149	182	78	74	87	91
	2008/9	43	40	51	68	178	187	77	96	84	95
CML441(S)×CZL00001(S)	2007/8	28	38	29	62	189	173	86	93	91	80
	2008/9	37	40	26	62	180	217	70	107	98	86
A1220-4(R)×B17(R)	2007/8	25	24	48	28	169	156	70	94	72	85
	2008/9	24	55	55	58	206	195	105	84	93	106
B17(R)×A15(R)	2007/8	30	27	40	44	186	180	92	68	49	101
	2008/9	48	56	74	64	185	184	96	113	122	84
A1220-4(R)×A15(R)	2007/8	18	21	33	57	232	145	119	87	78	112
	2008/9	48	34	49	58	233	172	129	87	92	125

†Sum of the number of individual plants scored for PLS severity. P_1 = the parent appearing first in each cross; P_2 = parent appearing second in each cross, $F_1 = (P_1xP_2)$, $F'_1 = (P_2xP_1)$; F'_2, $BCP_1xF'_1$ and $BCP_2xF'_1$ are the reciprocal crosses of F_1, F_2, BCP_1xF_1 and BCP_2xF_1 respectively. R = resistant, MR = moderately resistant and S = susceptible.

5.3 Data analysis

Mean disease ratings and variance of generations within each replication were calculated from individual plant ratings in the P_1, P_2, F_1, F'_1, F_2, F'_2, $BCP_1(F_1)$, $BCP_1(F'_1)$, $BCP_2(F_1)$ and $BCP_2(F'_1)$ generations using Genstat 12 (Payne *et al*., 2009). Frequency distribution curves of the F_2 and backcross generations derived from the crosses were plotted using the data analysis programme in Microsoft Excel 2007. The disease scores were transformed by square root (x + 1) to stabilize the treatment variances before analysis of variance. Data for each cross were analysed separately using PROC GLM procedure in SAS 9.1 (SAS Institute, 2002). The data were subjected to ANOVA firstly by environment with generation as the main effect, then a combined analysis across environments was conducted to analyse the effect of years, generations and interactions. Where significant differences between generations were observed, separation of means was carried out with the t- test ($P \leq 0.05$) and this was also used to detect differences in the F_1 reciprocal generations.

The ten generations for each cross were used to estimate the genetic effects using the following model (Kang, 1994):

$Y = m + \acute{a}a + \beta d + \acute{a}^2 aa + 2\acute{a}\beta ad + \beta^2 dd$

Where *m = mean, a = cumulative additive effect, d = cumulative dominance effect, aa = cumulative additive x additive effect, ad = cumulative additive x dominance effect, dd = cumulative dominance x dominance effect.*

The full model included interactions of the environment with each of the genetic effects. The full generation mean analysis (GMA) model was subjected to analysis in SAS following procedures described by Kang (1994).

5.3.1 Heritability estimates

Since individual plants were scored, variances among the plants within each generation were used to estimate generation variances. Broad-sense (H^2) heritability estimates were estimated using the equation:

$H^2 = 100*(\sigma^2_g / \sigma^2 F_2)$

Where: σ^2_g = genetic variance, $\sigma^2 F_2$ = total phenotypic variance of plants from F_2 generation.

The estimate of $\sigma^2 g = \sigma^2 F_2 - \sigma^2_e$, and σ^2_e = environmental variance = $(nP_1 s^2 P_1 + nP_2 s^2 P_2 + nF_1 s^2 F_1)/$ (Ne) (Wright, 1968); where n = the number of plants in each generation, $s^2 P_1$, $s^2 P_2$ and $s^2 F_1$ = variances of the Parent 1 (P_1), Parent 2 (P_2) and F_1 generations and Ne = $nP_1 + nP_2 + nF_1$.

Narrow-sense (h^2) heritability estimates were calculated using the equation:

$h^2 = 100*[2 \sigma^2 F_2 - (\sigma^2 BCP_1 + \sigma^2 BCP_2)]/ \sigma^2 F_2$ (Warner, 1952).

5.3.2 Minimum number of genes (effective factors)

The minimum number of genes controlling resistance to PLS in each cross was estimated using the formula (Wright, 1968):

$N = (X_1 - X_2)^2/8*(\sigma^2 F_2 - \sigma^2_e)$, where $\sigma^2 F_2 - \sigma^2_e = \sigma^2_g$

Where N = number of genes, X_1 = mean resistance of parent 1, X_2 = mean resistance of parent 2, $\sigma^2 F_2$ = variance of F_2 generation, σ^2_e = environmental variance within family.

The assumptions being that all the genes controlling the trait are unlinked; they affect the trait equally in size and direction; and there are no dominance or epistasis effects involved.

5.3.3 Heterosis

Mid-parent heterosis (MPH) was calculated as the performance of F_1 or (F'_1) compared with the average performance of its parents (Fehr, 1991). The following formula was used:

$MPH = 100*([M_{F1}-(M_{P1} + M_{P2})/2]/(M_{P1}+M_{P2})/2)$,

Where: M_{F1}, M_{P1} and M_{P2} are the mean disease severity scores for the parents (P_1 and P_2) and F_1 (or F'_1) generations, respectively.

5.3.4 Average degree of dominance

Average degree of dominance (ADD) was determined using the method of Mather and Jinks (1982), where:

$ADD = [M_{F1}-(M_{P1} + M_{P2})/2]/ (M_{P1}-M_{P2})/2$, where;

M_{P1}, M_{P2} and M_{F1} are the mean disease severity ratings in the parental and F_1 (or F'_1) generations, respectively.

5.4 Results

5.4.1 Disease development

In both 2007/8 and 2008/9 seasons, weather conditions were favourable for significant development of Phaeosphaeria leaf spot (PLS) disease by the soft dough stage. Mean ratings for the two seasons are shown in Table 5.4. Mean ratings for the susceptible parent CML441 were consistently high ranging from 6.8 to 9.0 in the different crosses where it was used. The second susceptible parent, CZL00001 had lower scores compared to CML441, ranging from 5.5 to 7.7. The third parent CML445 moderately resistant (MR) with scores ranging from 3.9 to 5.8. The resistant parents A1220-4, B17 and A15 all had lower scores in the two seasons, ranging from 2.2 to 3.8. In general, disease severity was higher in 2007/8 than in 2008/9 for most of the generations in the different crosses.

In the resistant or moderately resistant by susceptible crosses which involved CML441 as the susceptible parent, the two parental lines differed significantly as indicated by the t-test ($P \leq 0.05$, Table 5.5). However, in the MR x S cross that involved CZL00001 as the susceptible parent crossed to CML445, the two parental lines had similar reactions ($P > 0.05$) to PLS and both appeared to be more resistant than their progenies. For all the R x S and MR x S crosses indicated in Table 5.5, means of both the F_2 and F'_2 generations were not significantly different ($P \leq 0.05$) from the F_1 generation, but different

from the F'_1 reciprocal generation. The F_1 and F'_1 for crosses A1220-4 x CML441 and A15 x CML441 had a rating lower than the mean of the two parents. However, the F_1 and F'_1 of the MR x S crosses; CML445 x CML441 and CML445 x CZL00001 had a higher disease severity rating than the mean of the two parents.

Generally, in terms of ranking, means of the backcross generations to F_1 or F'_1 to either of the parents appeared closer to the recurrent parent except for the MR x S (CML445 x CZL00001) cross. There were no significant differences ($P \leq 0.05$) between the means of the two parental lines used in the R x R crosses (Table 5.6). However, in the S x S cross which involved CML441 x CZL00001, significant differences ($P \leq 0.05$) were observed between the means of the two parental lines, with CML441 being more susceptible than CZL00001 (Table 5.6). For the R x R crosses there was no distinct phenotypic segregation amongst the generation means of each cross, with all the generations having a mean disease severity score ranging from 2.8 to 3.9. The F_1 and F'_1 generations showed no significant differences from the parents in the cross A1220-4 x B17 but were significantly different from the parents in the crosses A1220-4 x A15 and B17 x A15 where they had a higher disease severity score.

All the generations in the S x S cross (CML441 x CZL00001) had ratings lower than the mean of the parents (Table 5.6). The mean disease severity scores for the generations ranged from 5.7 to 8.6. The F'_1 was significantly different from both parents, whereas the F_1 was different from only one parent (CML441). Both F_1 and F'_1 generations had lower mean scores than the parents.

Table 5.4 Mean disease ratings of generations based on individual plant ratings from the eight crosses evaluated for PLS severity at Cedara in 2007/8 and 2008/9 seasons

Cross	Year	Generations and mean disease ratings based on individual plant ratings†										LSD (0.05)[1]	Cross Mean	LSD (0.05)[2]	Env[3] Sig.
		P_1‡	P_2‡	F_1	F'_1	F_2	F'_2	BCP_1xF_1	$BCP_1xF'_1$	BCP_2xF_1	$BCP_2xF'_1$				
A1220-4 (R)[§] × CML441 (S)[§]	2007/8	2.4	6.8	3.4	3.0	3.9	3.9	3.0	2.7	4.1	4.9	**0.57**	**3.8**	**0.36**	**Ns**
	2008/9	2.2	8.7	3.6	2.8	4.1	4.1	2.8	2.6	3.9	2.7	**0.47**	**3.8**		
A15 (R) × CML441 (S)	2007/8	2.7	8.5	5.8	3.6	6.2	5.0	4.0	4.2	6.3	4.8	**0.57**	**5.1**	**0.39**	***
	2008/9	2.9	9.1	3.6	3.6	4.1	4.8	3.5	3.8	3.6	4.4	**0.55**	**4.3**		
CML445 (MR) × CML441 (S)	2007/8	5.3	9.0	7.0	6.3	6.5	6.4	5.9	5.1	7.3	7.0	**0.12**	**6.6**	**0.41**	***
	2008/9	3.9	8.7	7.4	6.8	6.1	5.9	5.8	5.0	5.3	5.1	**0.60**	**6.0**		
CML445 (MR) x CZL00001 (S)	2007/8	5.8	7.5	7.1	6.7	7.2	6.5	6.3	5.5	7.5	6.5	**0.44**	**6.7**	**0.35**	***
	2008/9	4.5	5.5	8.0	6.5	6.5	5.5	2.1	5.3	3.3	5.9	**0.54**	**5.3**		
CML441 (S) × CZL00001 (S)	2007/8	8.6	7.7	7.2	6.7	7.7	7.1	7.4	7.5	7.0	7.0	**0.52**	**7.4**	**0.43**	***
	2008/9	8.5	5.5	4.7	4.9	5.2	4.9	4.0	4.2	5.1	6.5	**0.70**	**5.4**		
A1220-4 (R) × B17 (R)	2007/8	3.0	3.8	3.9	3.8	3.6	4.2	3.5	3.4	4.4	4.6	**0.45**	**3.8**	**0.23**	***
	2008/9	3.4	2.7	2.7	2.6	2.8	2.8	2.7	3.0	2.8	2.8	**0.22**	**2.8**		
B17 (R) × A15 (R)	2007/8	3.5	2.9	3.7	3.7	4.0	3.5	3.6	3.6	3.5	3.4	**0.35**	**3.5**	**0.18**	***
	2008/9	2.7	3.2	3.1	3.0	3.3	3.3	3.2	3.1	3.5	3.2	**0.20**	**3.2**		
A1220-4 (R) × A15 (R)	2007/8	3.1	2.8	3.8	3.5	3.6	3.6	2.9	2.9	5.2	4.8	**0.55**	**3.6**	**0.28**	***
	2008/9	2.8	3.0	3.0	2.9	2.9	3.1	3.1	2.8	2.7	3.1	**0.28**	**2.9**		

†Means obtained from the total number of individual plants scored for PLS severity. ‡**P_1** is the parent appearing first in each cross and **P_2** is parent appearing second in each cross, $F_1 = (P_1 \times P_2)$ $F'_1 = (P_2 \times P_1)$; F'_2, $BCP_1 \times F'_1$ and $BCP_2 \times F'_1$ are the reciprocal generations of F_1, F_2, $BCP_1 \times F_1$ and $BCP_2 \times F_1$ respectively. [§]The resistance level of each parent is indicated by an R, MR or S. where R = resistant, MR = moderately resistant and S = susceptible. [1]LSD (0.05) = for comparing means for the generations within each cross for the year indicated. [2]LSD (0.05) is for comparing cross means for each cross for the the two years. [3]Env sig. = shows the differences between the two years for each cross as indicated by the ANOVA. NS = non significant differences between the two years.

Table 5.5: Generation means for PLS ratings of the R x S and MR x S crosses over two seasons

A1220-4 × CML441 (R x S)		A15×CML441 (R x S)		CML445×CML441 (MR x S)		CML445 ×CZL00001 (MR x S)	
Generation	**Mean**	**Generation**	**Mean**	**Generation**	**Mean**	**Generation**	**Mean**
P2 (CML441)	**7.7 A**	**P2 (CML441)**	**8.8 A**	**P2 (CML441)**	**8.8 A**	BCP2(F1)	7.7 A
F'2	4.0 B	F2	5.1 B	F1	7.2 B	F1	7.6 A
BCP2(F1)	4.0 B	F'2	5.0 B	F'1	6.7 B C	F2	6.8 A B
F2	4.0 B	BCP2(F1)	4.8 B C	BCP2(F1)	6.6 B C	F'1	6.6 B C
BCP2(F'1)	3.7 B	F1	4.7 B C D	F2	6.3 B C	BCP2(F'1)	6.2 B C D
F1	3.5 B	BCP2(F'1	4.5 B C D E	F'2	6.1 C	BCP1(F1)	6.1 B C D
BCP1(F1)	2.9 C	BCP1(F'1	3.9 C D E	BCP2(F'1	6.1 C	F'2	6.0 B C D
F'1	2.9 C	BCP1(F1)	3.9 D E	BCP1(F1)	5.9 C D	**P2 (CZL00001)**	**5. 9 C D**
BCP1(F'1	2.7 C D	F'1	3.6E	BCP1(F'1	5.1 D E	**P1 (CML445)**	**5.6 D**
P1 (A1220-4)	**2.3 D**	**P1 (A15)**	**2.7 F**	**P1 (CML445)**	**4.6 E**	BCP1(F'1)	5.4 D
Mean of the parents	**5.0**	**Mean of the parents**	**5.8**	**Mean of the parents**	**6.7**	**Mean of the parents**	**5.7**
LSD(0.05)	**0.36**		**0.39**		**0.41**		**0.35**

Means followed by the same letter for each cross are not significantly different at P=0.05. R and S = Resistant and susceptible, respectively.

Table 5.6 Generation means for PLS ratings of the R x R and S x S crosses over two seasons

A1220-4×B17 (RxR)		A1220-4×A15 (RxR)		B17×A15 (RxR)		CML441× CZL00001 (SxS)	
Generation	**Mean**	**Generation**	**Mean**	**Generation**	**Mean**	**Generation**	**Mean**
BCP2(F'1)	3.7 A	BCP2(F'1)	3.9 A	F2	3.5 A	**P1(CML441)**	**8.6 A**
BCP2(F1)	3.6 A B	BCP2(F1)	3.9 A	BCP2(F1)	3.5 A	BCP2(F'1)	6.9 B
F'2	3.5 A B	F1	3.3 B	BCP1(F1)	3.4 A	**P2(CZL00001)**	**6.6 B C**
P2(B17)	**3.2 A B**	F'2	3.3 B C	F'2	3.4 A	F2	6.4 B C D
F2	3.2 A B	F2	3.3 B C D	F1	3.4 A	BCP2(F1)	6.1 B C D
P1 (A1220-4)	**3.2 A B**	F'1	3.2 B C D E	F'1	3.4 A	F'2	6.0 B C D
F'1	3.2 A B	**P1 (A1220-4)**	**3.0 C D E**	BCP1(F'1)	3.3 A B	F1	5.8 C D
BCP1(F'1)	3.2 A B	BCP1 (F1)	2.9 C D E	BCP2(F'1)	3.3 A B C	BCP1(F'1)	5.8 C D
F1	3.2 A B	**P2 (A15)**	**2.9 C D E**	**P1(B17)**	**3.1 B C**	F'1	5.7 D
BCP1(F1)	3.1 B	BCP1 (F'1)	2.8 E	**P2(A15)**	**3.0 C**	BCP1(F1)	5.7 D
Mean of the parents	**3.2**	**Mean of the parents**	**2.9**	**Mean of the parents**	**3.1**	**Mean of the parents**	**7.6**
LSD (0.05)	**0.23**		**0.28**		**0.18**	**0.43**	

Means followed by the same letter for each cross are not significantly different at P=0.05. R and S = Resistant and susceptible, respectively.

5.4.2 Reciprocal crosses

Significant differences between the F_1 reciprocal generations were observed in the two R x S crosses and one MR x S cross (Table 5.5) and none for the R x R or S x S crosses (Table 5.6). In the R x S crosses (A1220-4 x CML441 and A15 x CML441), although both the reciprocal F_1 generations were resistant, the F'_1 (CML441 x A1220-4 and CML441 x A15) progenies were more resistant than the F_1 (A1220-4 x CML441 and A15 x CML441) progenies. A similar trend was observed for the MR x S cross, (CML445 x CZL00001), where both the reciprocal F_1 generations were susceptible, but the F'_1 (CZL00001 x CML445) progenies were less susceptible than the F_1 (CZL00001 x CML445). Generally, in all the other reciprocal F_1 generations (that is, both significant and non-significant), the F'_1 generation had lower disease severity mean scores than the F_1 generation.

5.4.3 Effect of years

Analysis of variance for the combined data showed generations to be significant ($P \leq 0.01$) for seven out of the eight crosses, with the exception of cross A1220-4 x B17 (R x R) (Table 5.7). Effects of years were significant ($P \leq 0.01$) for all the crosses with the exception of the cross A1220-4 x CML441 (R x S). Generation x year interactions were also significant ($P \leq 0.05$) for five of the eight crosses.

5.4.4 F_2 and backcross segregation

The distributions of the F_2 and backcross progeny scores for most of the crosses showed continuous variation (Fig. 5.1-5.3). The F_2 distribution varied from normal to slightly skewed either towards resistance or susceptibility depending on the cross. The backcross generations also followed the same trend. In general the R x S crosses had greater variation for both the F_2 and the backcross progenies (Fig. 5.1), whereas the R x R crosses (Fig 5.3) were more skewed towards resistance. The S x S crosses (Fig.5.2) had greater variation than the R x R crosses, with the distributions skewed towards susceptibility in 2007/8 season and more on resistance for F_2 and BCP_1 in 2008/9 and about normal for BCP_2 in 2008/9. Transgressive segregation towards either resistance or susceptibility was observed for some of the crosses as indicated in the distributions.

Table 5.7 Combined Analysis of variance for effects of years on the generation mean scores of eight maize crosses evaluated for PLS severity during 2007/8 and 2008/9 seasons at Cedara

Source of variation	df	A1220-4 (R) x CML441(S)	A15 (R) × CML441 (S)	CML445 (MR) × CML441(S)	CML445 (MR) × CZL00001 (S)	CML441 (S) × CZL00001 (S)	A1220-4 (R) × B17 (R)	B17 (R) × A15 (R)	A1220-4 (R) × A15 (R)
		Mean square	**Mean Square**	**Mean square**	**Mean square**	**Mean square**	**Mean Square**	**Mean Square**	**Mean Square**
Years	1	0.0043	0.3828**	0.1540**	0.1083**	1.7322***	0.7355***	0.0971***	0.3484***
Replication/years	2	0.0098	0.0669	0.0281	0.0052	0.0015	0.0127	0.0011	0.0029
Generations	9	0.4743***	0.4721***	0.2077***	0.0957***	0.1083***	0.0119	0.0081**	0.0415***
Generations × years	9	0.0439**	0.0673*	0.0265	0.0280	0.0494**	0.0254*	0.0093**	0.0438
Pooled Error	18	0.0091	0.0270	0.0145	0.0130	0.0126	0.0081	0.0026	0.0049***
R^2		0.97	0.92	0.90	0.84	0.93	0.88	0.85	0.93
CV (%)		5.05	7.67	4.81	4.52	4.46	4.95	2.77	3.90

*, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.0001, respectively. R, MR and S = Resistant, moderately resistant and susceptible, respectively

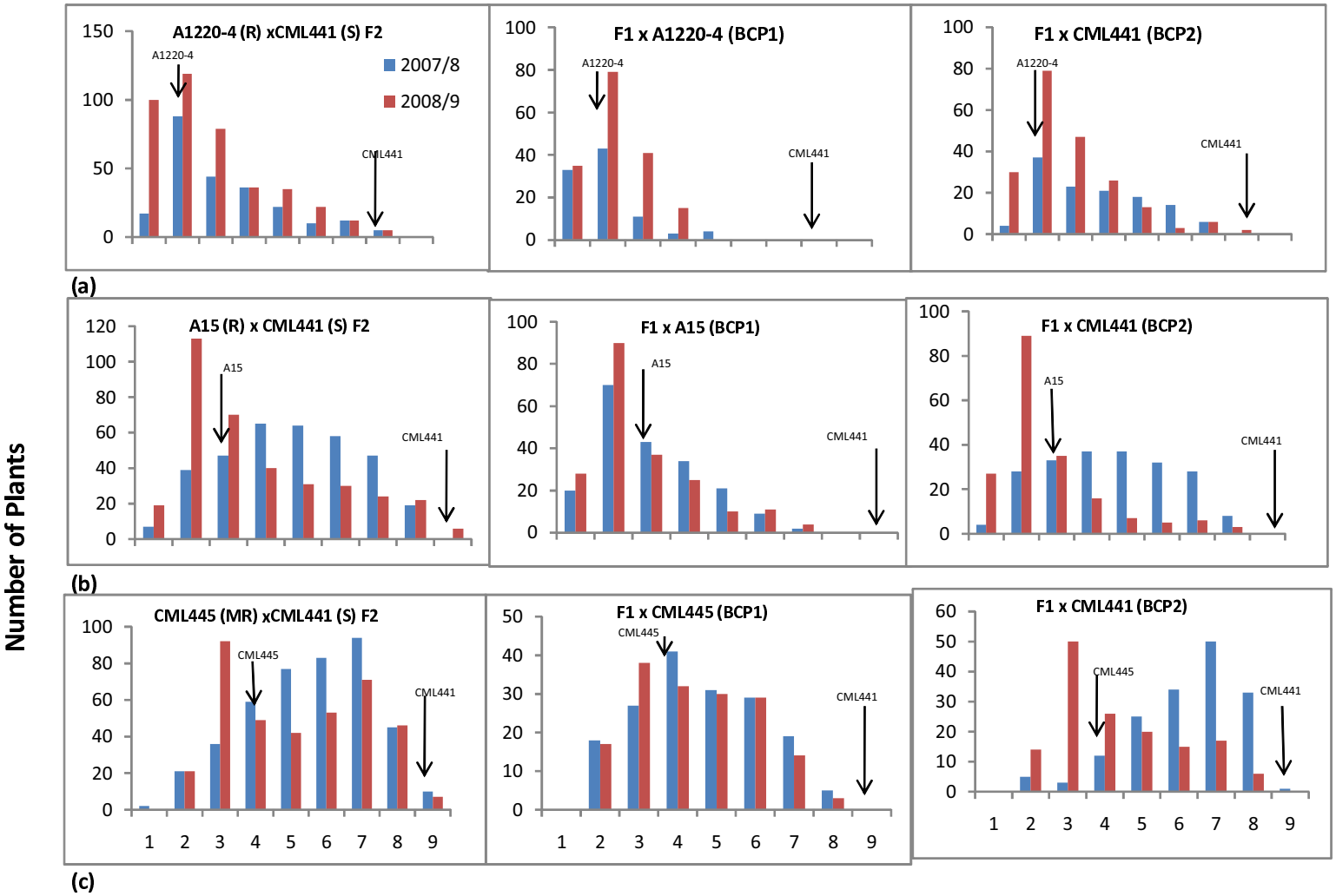

Figure 5.1 Frequency distributions of Phaeosphaeria leaf spot (PLS) ratings of individual plants of maize F_2 and backcross generations from the R x S crosses. The arrows point to the position of the different parents averaged across seasons.

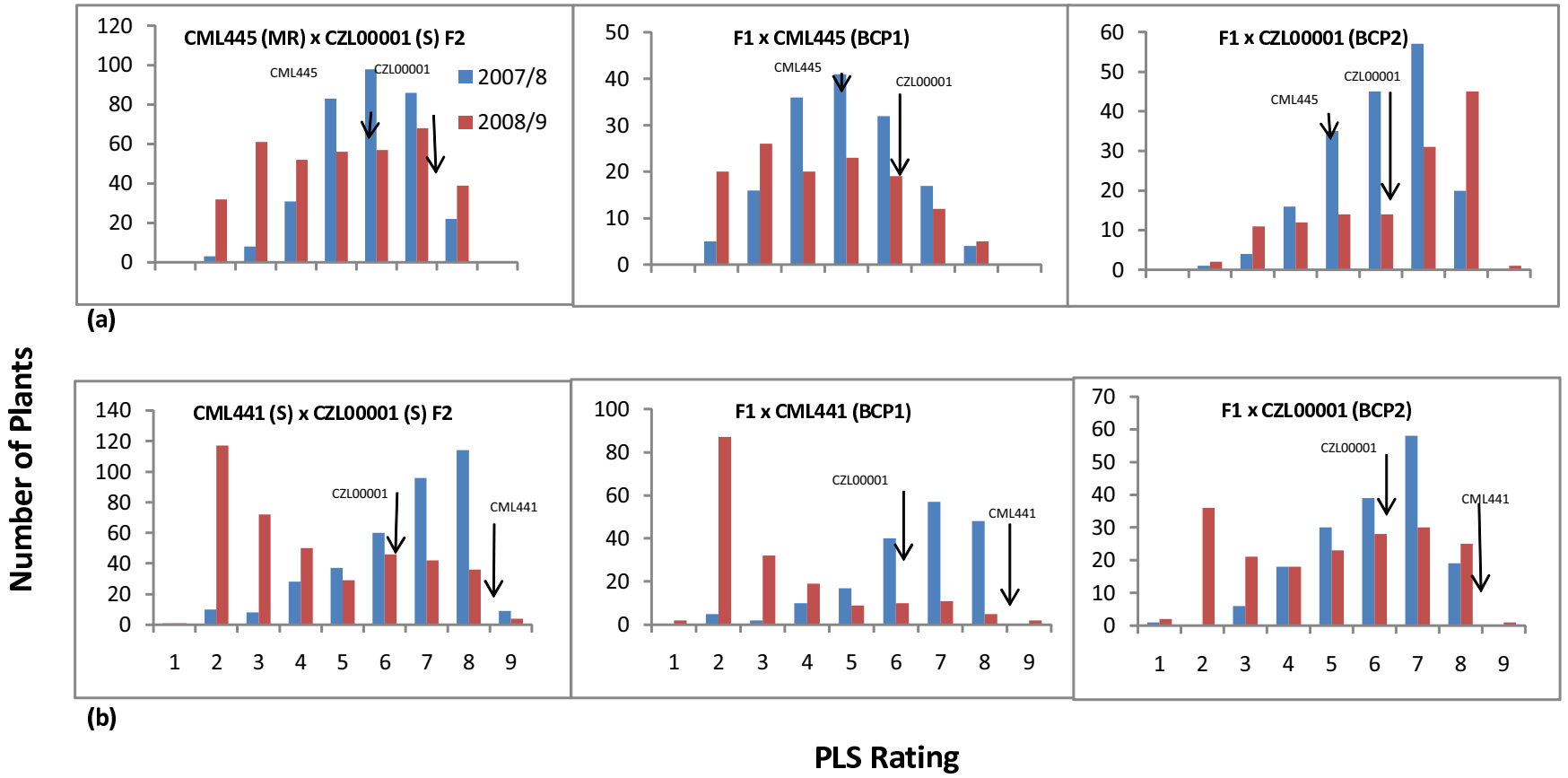

Figure 5.2 Frequency distributions of Phaeosphaeria leaf spot (PLS) ratings of individual plants of maize F_2 and backcross generations from the MR x S and S x S crosses. The arrows point to the position of the different parents averaged across seasons

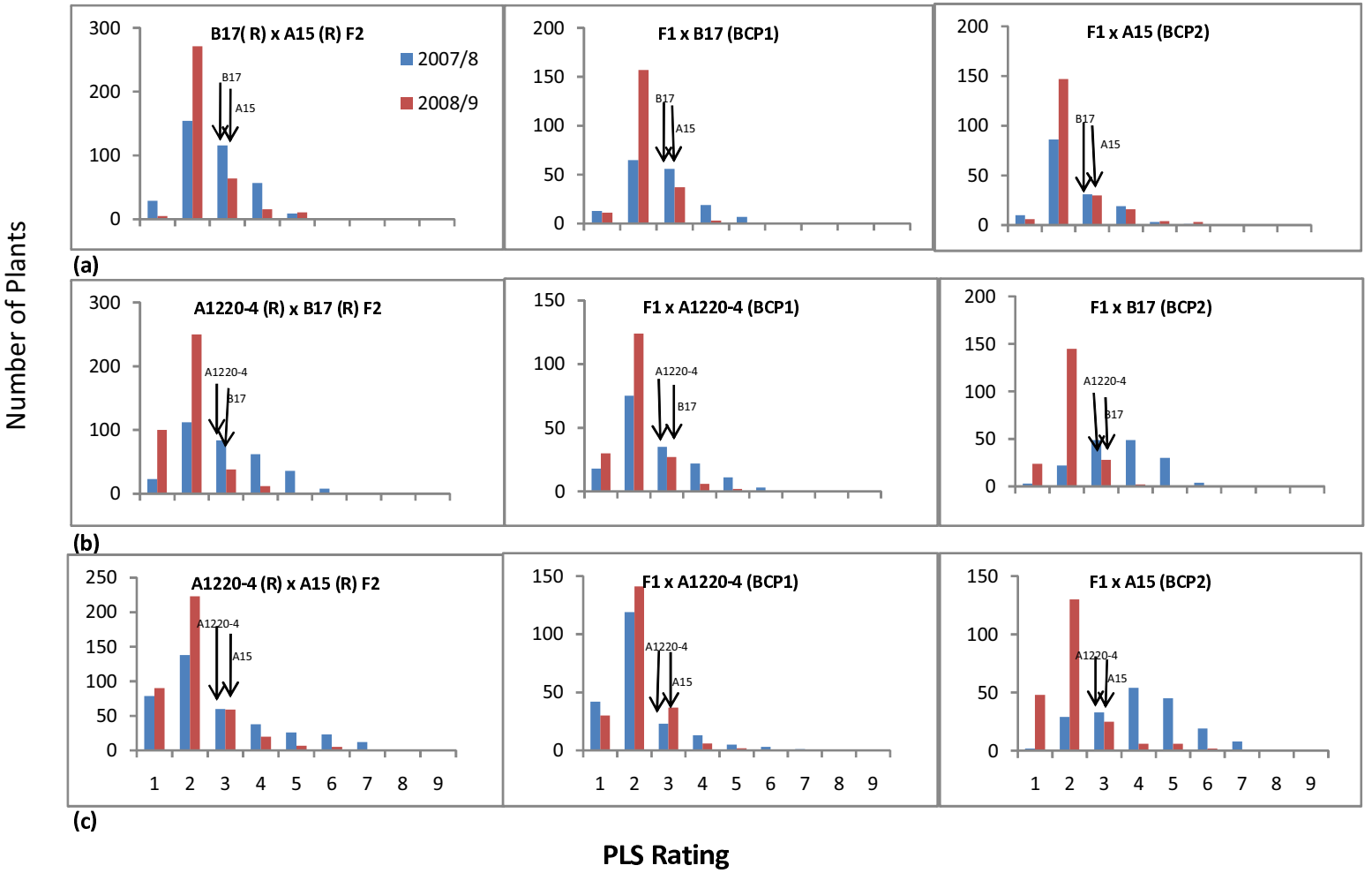

Figure 5.3 Frequency distributions of Phaeosphaeria leaf spot (PLS) ratings of individual plants of maize F_2 and backcross generations from the R x R crosses. The arrows point to the position of the different parents averaged across seasons.

5.4.5 Genetic effects

5.4.5.1 Resistant (R) or moderately resistant (MR) x susceptible (S) crosses

The estimates for additive effects (a) were significant (P≤0.05) and negative for all the crosses except for CML445 x CZL00001 (Table 5.8). Significant (P≤0.05) estimates for dominance effects (d) were observed for the two R x S and one MR x S crosses and these dominance effects were all negative and larger than their respective additive estimates. For these same crosses, non-allelic (epistasis) interaction effects were significant except for the additive x additive and dominance x dominance (dd) for the A15 x CML445 cross. The significant additive x additive estimates were all negative, whilst the dominance x dominance estimates were all positive. For CML445 x CZL00001 cross, only the additive x dominance interaction was significant (P≤0.01). The mid-point ranged from 2.03 to 3.06, being the lowest in the CML445 x CZL00001 cross.

The contributions to the total sum of squares (SSq) for the model were above 50% for additive effects in the three crosses which had CML441 as the susceptible parent (Table 5.9). Dominance effects contributed 3.5 to 11% of the total variation. The contribution of the additive x additive ranged from 6.8 to 9.4% and the dominance x dominance estimates contributed 2.11 to 6.0% of the variation. For the CML445 x CZL00001 cross, the dominance effect contributed 21% of the variation, with additive and additive x dominance contributing 6.8% and 8.6%, respectively.

5.4.5.2 Resistant x resistant (R x R) and susceptible x susceptible (S x S) crosses

Genetic effects for the R x R crosses were not significant, with the exception of the additive effects for A1220-4 x B17 and B17 x A15, which were significant (P≤0.05) (Table 5.8). The S x S cross had significant (P≤0.05) additive, dominance, additive x dominance and dominance x dominance effects (Table 5.8). The additive effects for the S x S cross were positive. The dominance effects were larger and negative than their respective additive estimates and the dominance x dominance were also positive. The mid-point for the S x S was 2.77 and for the R x R crosses it was much lower, ranging between 1.73 and 1.84. Contribution of the additive effects to the total SSq for the model in the S x S and R x R crosses ranged from 0 to 7% only, whereas the dominance effects ranged from 0 to 13%. The non-allelic interactions' contributed from 0 to 16% of the variation (Table 5.9).

Table 5.8 Estimates of genetic effects ±se

Model	A1220-4 (R) x CML441(S)	A15 (R) × CML441 (S)	CML445 (MR) × CML441 (S)	CML445(MR) × CZL00001(S)	CML441 (S) × CZL00001 (S)	A1220-4 (R) × B17 (R)	B17 (R) × A15 (R)	A1220-4 (R) × A15 (R)
m	2.83±0.30***	3.05±0.50***	3.06±0.31***	2.03±0.38***	2.77±0.28***	1.73±0.21***	1.79±0.12***	1.84±0.17***
a	-0.73±0.06***	-0.68±0.10***	-0.49±0.06***	0.08±0.078	0.28±0.06***	0.10±0.04*	-0.08±0.02***	-0.03±0.03
d	-2.79±0.72***	-2.48±1.18*	-2.10±0.76**	0.97±0.91	-1.44±0.68*	-0.17±0.49	0.19±0.28	-0.29±0.41
aa	-0.64±0.29*	-0.66±0.48	-0.61±0.31*	0.20±0.37	-0.13±0.27	-0.01±0.20	-0.08±0.12	-0.14±0.17
ad	1.10±0.21***	1.21±0.34***	-1.06±0.22***	-0.67±0.26**	-1.32±0.19***	-0.21±0.14	0.07±0.08	0.05±0.17
dd	1.72±0.44***	1.37±0.73	1.69±0.48***	-0.32±0.56	0.87±0.42*	0.05±0.31	-0.23±0.18	0.16±0.25

*, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.0001 respectively. R, MR and S = Resistant, moderately resistant and susceptible, respectively

Table 5.9 The relative contributions of the genetic effects to the total sum of squares (SSq) of the generation means in the different crosses

Source of variation	A1220-4 (R) x CML441(S) **%**	A15 (R) × CML441 (S) **%**	CML445(MR) × CML441 (S) %	CML445 (MR) × CZL00001 (S) **%**	CML441 (S) × CZL00001 (S) **%**	A1220-4 (R) × B17 (R) **%**	A1220-4 (R) × A15 (R) %	B17 (R) × A15 (R) **%**
a	64.25***	52.29***	54.73***	6.76*	1.88*	1.89	7.08***	0.07
d	11.19***	4.78**	3.56**	21.21***	13.58***	0.01	2.81**	10.69***
aa	2.13**	0.05	1.39	0.01	1.65*	1.28	1.89*	10.19***
ad	6.82***	9.47***	7.83**	8.63*	8.49***	3.54**	16.88***	0.02
dd	2.11**	3.41*	6.04***	0.05	1.50*	0.52	1.78*	0.65
R^2 for the Model	0.92	0.82	0.85	0.59	0.90	0.85	0.90	0.80
CV (%)	6.30	9.12	4.83	6.00	4.45	4.51	3.76	2.57

Significance based on the F-test: *, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.0001 respectively. R, MR and S = Resistant, moderately resistant and susceptible, respectively

5.4.6 Heritability estimates, minimum number of genes controlling PLS resistance, heterosis and average degree of dominance

Heritability estimates varied considerably amongst the crosses. Broad-sense heritability estimates for the R x S and MR x S crosses ranged from 41 to 83.7 % (Table 5.10), whereas narrow-sense heritability estimates were from 14 to 74.5%. In the S x S and R x R crosses, broad-sense heritability estimates ranged from 17 to 78% and narrow-sense heritability estimates from 0 to 45% (data not shown). The estimated minimum number of genes controlling PLS resistance ranged from less than one to four. Mid-parent heterosis ranged from 0 to 26.5% (Table 5.10) and the R x S crosses had negative heterosis (resistance), whereas the MR x S had positive heterosis (susceptibility). The degree of heterosis differed in the F_1 and F'_1 generations. Generally the R x S crosses had higher heterosis for the F'_1 than for their respective F_1 generations. In the S x S crosses, heterosis was towards resistance, whereas in the R x R crosses it was more towards susceptibility (data not shown).

The average degree for dominance (ADD) for resistance in the R x S and MR x S crosses which had CML441 as the susceptible parent, varied from -0.07 to 0.7, depending on whether the F_1 or F'_1 was used. However, values for the MR x S (CML445 x CZL00001) cross and all the R x R and S x S crosses were more than +1 or less than -1.

Table 5.10: Estimates of broad-sense heritability (H^2), narrow-sense heritability (h^2), minimum number of genes (MNG), mid-parent heterosis and average degree of dominance (ADD) of resistance to Phaeosphaeria leaf spot in maize for the R x S and MR x S crosses

Cross	Year	H^2 (%)	h^2 (%)	MNG	Mid-parent heterosis (%)		ADD	
					F1	F'1	F1	F'1
A1220-4 (R) x CML441 (S)	2007/8	82.7	70.8	1.8	-11.4	-17.7	0.45	0.70
	2008/9	83.5	74.5	3.5	-14.2	-24	0.43	0.73
	Combined	72.8	71.1	2.7	-11.9	-20.4	0.41	0.71
A15 (R) x CML441 (S)	2007/8	80.8	40.0	3.1	9.1	-19.2	-0.32	0.60
	2008/9	84.0	54.4	2.4	-19.2	-18.5	0.67	0.64
	Combined	73.9	68.4	3.0	-6.2	-18.1	0.27	0.63
CML445 (MR) x CML441 (S)	2007/8	74.9	38.7	2.1	-5.4	0.02	0.41	-0.001
	2008/9	83.7	52.7	1.7	6.2	10.3	-0.31	-0.52
	Combined	62.8	40.4	2.6	1.2	5.6	-0.07	-0.33
CML445 (MR) x CZL00001 (S)	2007/8	49.4	14.1	0.7	0.5	4.1	-0.04	-0.02
	2008/9	71.2	21.7	0.1	14.3	26.5	-2.64	-4.90
	Combined	41.1	15.1	0.3	7.6	15.4	-1.29	-2.61

5.5 Discussion

5.5.1 Disease development

Disease rating was done at the hard dough stage and this rating closely reflected the total damage to the leaf tissue for the entire growing season. Although the disease pressure seemed to be different in the two seasons, it was high in both seasons as indicated by the generally high PLS scores recorded for the most susceptible parent, CML441. The susceptible parents differed in the degree of susceptibility, with CML441 being more susceptible than CZL00001 to PLS, indicating that although both susceptible, the genetic backgrounds are different. CML445 which had been selected on the basis of being PLS susceptible (Derera *et al*., 2007) turned out to be moderately resistant despite the disease pressure being high and was therefore reclassified to moderately resistant. There was good significant differentiation between parents involved in the R x S or MR x S crosses with CML441 as the susceptible parent. This pattern of response, indicated that differences observed in disease severity among the generations were due to genetic differences among parents. However, the MR x S cross between CML445 x CZL00001 did not show any significant differences between the parents. The result also indicated that PLS resistance in CZL00001 was moderately susceptible to susceptible. It appears, therefore, that CML445 might be classified as moderately resistant or moderately susceptible. Disease pressure and the environment seem to influence the classification. In this study, the ratings of CML445 ranged from 3.9-5.8, whereas in studies by Derera *et al*. (2007), the score averaged 6.0 at Rattray Arnold Research Station (RARS) in Zimbabwe. In addition, Derera *et al*. (2007) observed a positive general combining ability for CML445 when it was used as the female line, suggesting it contributed susceptibility in most hybrids it was involved. The similarity between CML445 and CZL00001 was not ideal for the generation mean analysis which requires that the parents be diverse in the trait being studied.

5.5.2 Effect of years and frequency distributions

Year effects were significant for seven out of eight crosses and there was significant generation x year interaction. The interaction could have been a result of the generally high generation mean scores for most of the crosses in 2007/8 season than 2008/9 (Table 5.4). In addition, there were some changes in the magnitude of the differences between the two parental lines used in the R x S or MR x S crosses. In general, there were some slight significant changes in the ranking of the generations in some of the crosses.

Variation in the segregating populations was approximately continuous and normal to slightly skewed for some of the crosses. This was, however, consistent with quantitative inheritance and the observation made by Carson (2001) for PLS in the B73 x Mo17 cross. Apparent transgressive segregation was observed in the MR x S, S x S and R x R crosses towards both resistance and susceptible. It may, therefore be possible to identify plants with good resistance from crosses between MR x S and S x S lines. A higher level of resistance can also be expected from crosses between R x R lines. The distributions also indicated that several loci controlled the inheritance of PLS resistance.

5.5.3 Heterosis and average degree of dominance (ADD)

The F_1 and F'_1 generations for the A1220-4 x CML441 and A15 x CML441 had lower mean disease ratings than the mean of the two parents, indicating existence of heterosis towards resistance. This was further confirmed by the mid-parent heterosis values which were also negative indicating resistance. Derera (2005) reported relatively high mid-parent heterosis values for the R x S and MR x S crosses, ranging from 4 to 53% towards resistance. It seems, therefore, that for PLS resistance in maize, populations differ in terms of the dominance effects. The F_1 and F'_1 generations of the MR x S crosses (CML445 x CML441 and CML445 x CZL00001) in this study had a higher disease severity rating than the mean of the two parents, and their mid-parent heterosis values were positive indicating heterosis towards susceptibility. Heterosis can be expressed when parents of a hybrid have different alleles at a locus and there is some level of dominance, overdominance or epistasis among these alleles (Falconer and Mackay, 1996).

The average degree of dominance values in the R x S crosses in which the parents demonstrated a wide significant difference, ranged from -0.001 to 0.73. Since a value of 1 or -1 is considered to indicate complete dominance (Edwards and Lamkey, 2002), it means that for PLS, in these crosses, the genes controlling resistance exhibit incomplete dominance. In the crosses (S x S; R x R) where the differentiation between parents was insignificant, values for ADD were much higher than 1 or -1. It seems in such crosses dominance or even over-dominance could be important. These results were also corroborated by the contributions of the genetic effects to the total variation among generations, which were much higher in these crosses than for the additive effects.

5.5.4 Reciprocal crosses

When the resistant lines were used as maternal parents, the F_1 progeny although resistant, was not as resistant as when they were used as paternal parents. It seems, therefore, that the susceptible parent in these crosses, CML441 contributed some genes for resistance which could be associated with the cytoplasm. The other MR x S cross, CML445 x CZL00001 with significant reciprocal F_1 generations followed a similar trend. Although both the F_1 reciprocal generations had mean disease scores classified as susceptible, the resistant parent when used as the maternal parent resulted in progeny with more disease than when used as the paternal parent. The occurrence of cytoplasmic effects for PLS has not been reported before. The only report of suspected cytoplasmic inheritance was by Derera *et al*. (2007) based on the predominance of the female GCA over the male GCA. It appears that CML441 contributed resistance as a female parent, suggesting that it has maternal effects in favour of resistance and CZL00001 showed a similar effect

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Investigation of maternal effects is important because for a trait that is completely under maternal effects (that is, cytoplasmic or genetic), the amount of genetic variance would be inflated and this tends to slow the response to selection (Roach and Wulff, 1987; Hallauer and Miranda, 1988). In addition, presence of maternal effects influences the choice of female line in single cross hybrids, that is, the female should be the resistant line, if the levels of resistance are to be enhanced. The observation made in this study has some implications in designing hybrids for deployment in the small-scale farming sector in sub-Saharan Africa (SSA). The result showed clearly that resistance would be enhanced in F_1 hybrids when the more susceptible parents CML441 and CZL00001 were used as female. Usually breeders would use the resistant parent as the female/seed parent to enhance seed yield, but this would compromise F_1 hybrid resistance to PLS.

5.5.5 Genetic effects

The models indicated a very good fit for most of the crosses as indicated by the high R^2 values. The R x S crosses, with the exception of the MR x S (CML445 x CZL00001), had predominantly additive gene action. More than 50% of the total variation was due to additive gene effects, confirming that resistance to PLS was predominantly additive as reported by other investigators (Carson, 2001; Silva and Moro, 2004; Mhembere, 2005; Derera *et al*., 2007; Vivek *et al*., 2009). These significant additive genetic effects imply that selection for increased PLS resistance should be effective and the performance of the offspring predictable on the basis of the reaction of parents (Carson, 2001; Derera *et*

al., 2007). However, for the MR x S (CML445 x CZL00001) cross, only the additive x dominance genetic effects were significant. Carson (2001) reported significant but less important dominance effects and no epistasis in the cross B73 x Mo17. Vivek *et al*. (2009) also reported both significant GCA and SCA effects to be significant, with a contribution of 65% and 35%, respectively, to the total genetic variation. Effects of GCA describe additive gene action, whilst SCA deals with other types of gene action. However, Derera *et al*. (2007) reported significant GCA effects and non-significant SCA effects for resistance to PLS suggesting only additive gene action was at play in the crosses evaluated. In this study, the R x S crosses resulted in significant negative dominance genetic effects, an indication that dominance was important in the mechanism for resistance to PLS. Nevertheless, the contribution of dominance effects to the total variation was relatively small, ranging between 3-11% and was within the range reported by Carson (2001) for the B73 x Mo17 cross. This implies that, although significant and important, dominance has a much smaller contribution to PLS resistance in maize than additive gene effects. Dominance can be exploited in hybrid production.

There was also an indication of epistatic gene action in the mechanism of PLS resistance in the populations studied. The significant negative additive x additive estimates as well as the significant positive dominance x dominance effects observed for the R x S and MR x S crosses suggested that directional epistasis was present and this is consistent with findings by Rodriguez- Herrera *et al*. (2000). In this study, while the sign for dominance was negative, the sign for dominance x dominance was positive indicating existence of duplicate types of gene interactions, thus confirming the importance of dominance effects as indicated by Mather and Jinks (1982) and Grewal (1988). The dominance x dominance contributed towards more disease. The additive x dominance effects were also highly significant and seemed to be more important than the additive x additive in the inheritance of PLS resistance. The additive x dominance effects could be important in heterosis as suggested by Kearsey and Pooni (1996). These results show that PLS resistance may be the result of many different loci, with both additive and dominance effects as well as epistasis being important factor in the resistance.

Presence of epistasis and dominance can affect heritability estimates and number of effective factors (genes) (Fernandez and Miller, 1985). Therefore in this study the heritability estimates and number of genes observed could have been affected downwards by the presence of both dominance and epistasis in most of the populations. According to Hayman (1960), presence of epistasis is of major importance in the inheritance of a trait and results in biased estimates of pooled additive and dominance

effects. The significant negative additive x additive effects show that gene pairs for resistance are in dispersive form as suggested by Mather and Jinks (1982) and Kearsey and Pooni (1996). This means that both parents contributed genes for PLS resistance, that is, there was non-directional distribution of alleles in the two parents agreeing with reports by Gelner and Sechler (1986) and Kearsey and Pooni (1996). However, the result of this study was in contrast with the observation made by Carson (2001) for the B73 x Mo17 cross, where they concluded that only one parent contributed genes for PLS resistance. According to Zwedie and Bosland (2003) when gene pairs are in dispersive form, estimates of the number of effective factors would be low. Therefore, the presence of gene pairs in dispersive form observed in this study could have affected the estimates of effective factors in some of the populations.

5.5.6 Heritability estimates and minimum number of genes (effective factors) controlling PLS resistance

The heritability estimates for the R x S crosses indicated that PLS resistance was moderate to highly heritable and also indicated the degree of the differences between the parental lines in these crosses. The broad-sense heritability estimates ranged from 62 to 84% and were within the range of heritability estimates reported for PLS in other studies (Carson, 2001; Mhembere, 2005; Derera *et al*., 2007). The narrow-sense heritability estimates in this study ranged from 40 to 74% and were slightly lower than the 70 to 85% reported by Carson (2001). The presence of dominance and epistasis could also have affected the estimates of heritability downwards. Where the parental lines were not significantly different, that is, S x S and R x R crosses, heritability estimates were lower. This could be as a result of less genetic variation or phenotypic segregation within the different generations. Differences in heritability estimates amongst different studies could be a result of differences in either the environment and/or the genotypes used as well as different levels of additive variance versus dominance and epistatic variances (Falconer and Mackay, 1996). Heritability estimates seem to increase with larger disparities between parents, which results in wide genetic variation and this observation agreed with the findings of Van Ginkel and Scharen (1987).

However, the relatively medium to high estimates of heritability observed in this study indicate that a substantial portion of the genetic variation is additive in nature. These results were consistent with the observation of less than 11% dominance and epistatic effects in the R x S and MR x S crosses. Therefore, improvement of PLS resistance can be realized through breeding as suggested by Carson (2001) and Derera *et al*. (2007).

High additive variance and narrow-sense heritability indicate that conventional pedigree and early generation selection could be effective for initial improvement of PLS resistance in maize.

Most of the R x S crosses had minimum number of genes between one and four. The MR x S cross (CML445 x CZL00001) had zero to one resistant gene, whereas the other MR x S involving CML445 x CML441 had 2-3 genes. It seems CML441; though susceptible contributes to PLS resistance in the crosses it is involved. The observation was also supported by the results of the reciprocal crosses for A1220-4 x CML441 and A15 x CML441 where more resistant progeny were observed when CML441 was used as the maternal parent, suggesting large maternal effects in favour of resistance. The estimates of the number of genes in this study were close to what Carson (2001) observed in the study of generations made from B73 x Mo17. On the other hand, Pegoraro *et al*. (2002) using Brazilian lines reported two major independent genes that were involved in the inheritance of resistance to PLS disease. However, dominance and most types of epistasis have been reported to bias estimates of effective factors downwards (Wright, 1968). It is possible therefore that the number of effective factors could be more than what was observed in this study, but were biased by the failure to meet the assumptions in the analysis of variance of no epistasis and no dominance in the R x S crosses where the epistasis effects were significant.

5.6 Conclusion

In this study, parental lines selected for the R x S crosses varied widely in their resistance to PLS except for CML445 which had to be reclassified to moderately resistant. The inbred lines used in this study differed in their susceptibility levels. However, the resistant lines A15, B17 and A1220-4 appeared to have similar genes for resistance as there were no significant differences between the parents in the different R x R crosses. The R x S crosses also confirmed that resistance to PLS in the inbred lines used was predominantly controlled by genes with additive effects, but with both dominance effects and epistasis being important factors in the resistance. The significant negative additive x additive effects showed that gene pairs for PLS resistance in these inbred lines were in dispersive form, that is, both parents contributed genes for resistance. The susceptible CML441 inbred line produced F_1 progeny that was more resistant when it was used as a maternal parent in R x S crosses. This implied that it had maternal effects or cytoplasmic genes in favour of resistance and CZL00001 also showed a similar effect. Resistance to PLS was moderate to highly heritable. Apparent transgressive segregation was observed in the

MR x S, S x S and R x R crosses towards both resistance and susceptible. It may, therefore be possible to identify plants with good resistance from crosses between MR x S and S x S lines. A higher level of resistance can also be expected from crosses between R x R lines. The frequency distributions for the F_2 and backcross progenies also indicated that several loci controlled the inheritance of PLS resistance. Mid-parent heterosis values for the R x S crosses were negative indicating heterosis towards resistance. The average degree of dominance values in the R x S crosses indicated that, the genes controlling PLS resistance exhibit incomplete dominance. Most of the R x S crosses had minimum number of genes between one and four, the MR x S crosses (CML445 x CZL00001) had zero to three genes.

Highly significant additive effects and moderate to high heritability estimates imply progress can be made through selection, although selection could be slowed by epistasis in these populations. Dominance effects which were associated with reduced disease levels may also be exploited in maize hybrid production. In addition, the results indicated that resistance would be enhanced in single cross hybrids when susceptible parents CML441 and CZL00001 are used as female, although this may have serious implications on seed yield.

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6 Generation Mean Analysis and Combining Ability for Grey Leaf Spot Resistance in Elite African Maize Germplasm

Abstract

Maize grey leaf spot (GLS) remains an important foliar disease in sub-Saharan Africa. More information on the combining ability for GLS resistance of germplasm adapted to African environments is required in new sources being identified. In addition, it is not known whether the simple additive-dominance model for GLS resistance can be applied to the African germplasm as the non-additive gene action from the SCA effects has not been partitioned. This study was conducted to determine the combining ability and the types and magnitude of gene action for resistance to GLS among selected tropical advanced maize inbred lines. Forty five F_1 hybrids generated by crossing ten inbred lines in a half diallel mating scheme were evaluated in six environments, with two replications each between 2007 and 2009. Reciprocal crosses and backcross progenies were also generated among inbreds A1220-4, A15, CML441 (resistant, R), and N3 and B17 (susceptible, S). These were evaluated for GLS resistance in two replications at Cedara Research Station in South Africa and the data subjected to generation mean analysis (GMA) in SAS. General combining ability (GCA) and specific combining ability (SCA) effects were highly significant ($P \leq 0.001$), with GCA effects accounting for 71% and SCA effects 29% of the (cross) hybrid sum of squares for GLS resistance, thus indicating the predominance of additive over non-additive gene action. The most resistant inbred lines were A1220-4, CZL00009, CZL00001, CML205 and CML443. These lines had good combining ability for GLS resistance and contributed towards resistance in their respective crosses. Generation mean analysis showed the additive effects to be highly significant ($P \leq 0.001$) and contributing >89% of the total variation due to generations. Dominance effects in cross A15 x B17 accounted for 7% of the variation, while epistasis was observed for crosses CML441 x N3 and A1220-4 x B17. No reciprocal differences in the F_1 hybrids were detected for the three crosses, suggesting that maternal effects did not play a major role. Resistance was highly heritable (54-92%) and controlled by genes exhibiting no dominance to partial dominance with the involvement of two to three genes. Overall, highly significant additive effects and high heritability estimates imply that progress would be made through selection, although the significant epistasis and dominance which compromised heritability in some of the populations could slow progress to selection. Dominance effects which were associated with reduced disease levels may also be exploited in developing single cross maize hybrids among these inbreds when one of the parents is resistant.

6.1 Introduction

Maize grey leaf spot (GLS) is currently one of the most important foliar disease in sub-Saharan Africa (Menkir and Ayodele, 2005; Vivek et al., 2009). The disease results in yield losses of around 10 to 25% annually, but losses as much as 90% due to severe deterioration of the leaves and stalk lodging have also been recorded (Latterell and Rossi, 1983). Management of GLS disease has focused mostly on the use of fungicides and genetic resistance (Gordon *et al*., 2006), but farmers in sub-Saharan Africa (SSA) have limited access to chemicals. This makes control of GLS in the smallholder sector difficult when susceptible varieties are grown. Therefore, it would be practical to breed varieties with acceptable levels of GLS resistance to minimize grain yield losses.

Diverse sources of GLS resistance and the genetic basis of resistance have been identified and reported by many researchers (Thompson *et al*., 1987; Huff *et al*., 1988; Donahue, *et al*., 1991; Gevers *et al*., 1994; Coates and White, 1998; Menkir and Ayodele, 2005; Pratt and Gordon, 2006; Derera *et al*., 2008; Vivek *et al*., 2009). However, the majority of these studies have been conducted on temperate materials which cannot be used directly in African tropical environments. Only a few sources of resistance have been identified from some African adapted germplasm. Gevers *et al*. (2004) observed high resistance in some white modified *opaque*-2 maize (KO54W and SO507) belonging to the F and M heterotic groups, respectively in South Africa, but these are not adapted to tropical conditions in SSA. In addition, Derera *et al*. (2008) also identified some resistant sources in heterotic groups A, N3, B, K and SC which are adapted to tropical conditions. However, given the potential of GLS to threaten food security, more sources of resistance would be useful, especially those that can contribute resistance to inbred lines that are susceptible to GLS and are widely used in hybrid production in Africa. In addition, information is still limited on the mode of inheritance for most of the germplasm that are adapted to African environments (Derera *et al*., 2008).

Resistance to GLS has been shown to be controlled mainly by additive genetic effects (Thompson *et al*., 1987; Huff *et al*., 1988; Ulrich *et al*., 1990; Gevers and Lake, 1994; Derera et al., 2008; Vivek *et al*., 2009), although dominant gene action has also been shown to play a role (Elwinger *et al*., 1990; Coates and White, 1998; Derera *et al*., 2008; Vivek *et al*., 2009). Hohls *et al*. (1995) reported that resistance was conditioned by additive and complete dominance with minor epistasis in a diallel comprising maize lines from three divergent backgrounds in South Africa. Predominance of additive gene action

suggests breeding for resistance would be easy as resistance in regional maize can be enhanced by selection in hot spot environments.

Several studies have been conducted to determine the inheritance of resistance to GLS in diverse sources of maize inbred lines, with some suggesting the possible role of cytoplasmic effects. For example, independent studies by Derera (2005) and Menkir and Ayodele (2005) reported large differences between male and female mean squares for GLS resistance. The studies also showed that single cross hybrids would be resistant when at least one of the inbred lines carried the resistance to GLS (Derera, 2005; Menkir and Ayodele, 2005). If cytoplasmic effects exist, then the choice of the female line in a single cross between a susceptible and resistant line would be critical. Nonetheless, detailed studies on cytoplasmic influences have not been done. The role of cytoplasmic effects in the inheritance of resistance to GLS needs investigating using models that include reciprocal effects (Roach and Wulff, 1987). Differences in reciprocal crosses have been used as the most direct evidence for unequal contribution by maternal and paternal parents to the phenotype of offspring (Roach and Wulff, 1987). Reciprocal pairs have similar nuclear genetic contribution and any difference in performance of reciprocal pairs will be due to maternal (or perhaps paternal) gene effects (Cockerham and Weir, 1977; Roach and Wulff, 1987).

Diallel mating design was chosen as a method as it enables analysis of crosses among a group of parents including the parents themselves and the estimation of general combining ability (GCA), specific combining ability (SCA) and other effects (Jinks and Hayman, 1953; Hayman, 1954; Griffing, 1956). It, therefore, provides an assessment of important qualities of the parents which may be useful in selection and development of new germplasm. Although GCA describes additive gene effects, SCA describes all the non-additive genes effects, that is, dominance and epistasis. On the other hand, generation mean analysis (GMA) provides a detailed analysis of a cross. Methods have been developed for GMA to separate the epistatic variation from the additive and dominance variation (Hayman, 1958, Gamble, 1962). In addition, for GMA, the populations used provide generations that can be used in a breeding program (Coates and White, 1998).

This study was therefore conducted to i) determine the combining ability for resistance to GLS among selected maize inbred lines using a diallel analysis, ii) determine the types and magnitude of gene action for resistance to GLS from resistant and susceptible inbred

parents using generation mean analysis, and iii) investigate the existence of cytoplasmic gene effects in the inheritance of GLS resistance in tropical African maize lines.

6.2 Materials and methods

6.2.1 Maize germplasm and diallel crosses

Maize inbred lines used were obtained from the CIMMYT programme in Harare, Zimbabwe, while the inbred N3 was obtained from the Crop Breeding Institute in Zimbabwe. The inbred lines used for the diallel and generation mean analyses are indicated Table 6.1. Standard hybrid checks included were selected on the basis of their grain yield performance, stability and also reaction to a number of foliar diseases mainly phaeosphaeria leaf spot (PLS), GLS, northern corn leaf blight (NLB) and common rust. Hybrids that are commonly grown by resource-poor smallholder farmers in the region were also included.

Table 6.1 Designation, pedigrees and heterotic groups for parent inbred lines used in the diallel analysis

Designation†	Pedigree or Population (OPVs)	Heterotic grouping	Selection criteria	Experiment‡
A1220-4	[(CML395/CML444)-B-4-1-3-1-B/CML395//SC/ZM605#b-19-2-X]-1-2-X-1-1-BBBBBB]-7-1-3-2-BBB	B / SC	PLS susceptible	D and GMA
CML205	[EMSR]#B#bF101sr-2-1-sr-3-2-4-b-b	B	GLS susceptible	D
A16	Original pedigree CML312 (S89500F2-2-2-1-1-B*5)	A	GLS susceptible	D
CML445	[[TUXPSEQ]C1F2/P49-SR]F2-45-7-5-1-BBB	AB	PLS susceptible	D
CML488	DTPWC8F31-4-2-1-5-BBB	AB	PLS resistant	D
CZL00001	INTA-191-2-1-2-BBBB	A	PLS susceptible	D
CZL00009	INTA-F2-192-2-1-1-1-BBBBB	A	PLS susceptible	D
MP18	[[[NAW5867/P30SR]-111-2/[NAW5867/P30SR]-25-1]-9-2-3-B-2-B/CML388]-B- 35-2-B-1-#-1-BB	A / P	GLS susceptible	D
N3	Salisbury White	N3	GLS susceptible	D and GMA
CML443	[AC8342/IKENNE{1}8149SR//PL9A]C1F1-500-4-X-1-1-BB-1-BB	AB	GLS resistant	D
A15	[CML197/N3//CML206]-X-32-1-4-B-B-B-B	A / N3	GLS resistant	GMA
B17	[LZ956441/LZ966205]-B-3-4-4-B-5-B-B-B-B	B	GLS susceptible	GMA
CML441	ZM605C2F1-17-1-B-1-BB	B	GLS resistant	GMA

†some of the lines like A1220-4, A16, MP18, A15 and B17 were coded for convenience of study. ‡D = diallel analysis, GMA = generation mean analysis

The ten advanced maize inbred lines were crossed in a full diallel mating scheme (excluding selfs) in 2006/7 season at the University of KwaZulu-Natal (UKZN), Pietermaritzburg, South Africa. The resulting F_1 single cross hybrids and their reciprocals

were harvested and the seed initially kept separately, but later bulked after selecting F_1 reciprocal crosses for the generation mean analysis.

6.2.2 Generation mean analysis

The inbred parents used for the generation mean analysis were three resistant (R); CML441 (R1), A1220-4 (R2) and A15 (R3) and two susceptible (S); N3 (S1) and B17 (S2). The F_1 single crosses included the following R_1 x S_1; R_2 x S_2; R_3 x S_2. These classifications based on the disease reaction will be referred to as a cross in this study. The other crosses made from the F_1 single cross hybrids will be referred to as generations.

The F_1 single cross hybrids and their reciprocals (F'_1) were self-pollinated and backcrossed to each of the parents to produce F_2 , $BCP_1(F_1)$, and $BCP_2(F_1)$ plus their reciprocal generations (F'_2 , $BCP_1(F'_1)$ and $BCP_2(F'_1)$), where P_1 was the first parent and P_2 the second parent in each cross. These generations were produced during winter of 2007 at UKZN, Pietermaritzburg in South Africa.

6.2.3 Field evaluations for diallel analysis

The 45 single cross F_1 hybrids plus nine standard checks were evaluated in 2007/8 and 2008/9 seasons in a total of six environments. The parents plus four inbred checks were also evaluated in trials adjacent to the hybrid trials, but only in three environments. The six environments included: Cedara (C), South Africa (30°16'E, 29°32'S, 1130 metres above sea level (m.a.s.l); Baynesfield (BF) Estate, South Africa (30°21'E, 29°46'S, 758 m.a.s.l); Rattray Arnold Research Station (RARS), Zimbabwe (31°14'E, 17°40'S, 1300 m.a.s.l), and Mpongwe, Zambia (ZAMB) (28°8'E, 13°31'S, 1219 m.a.s.l). At Cedara, plantings were done in November 2007 (C108), January 2008 (C208) and November 2008 (C09). Plantings at RARS were in December, 2007 (RARS08); Mpongwe, January 2008 (ZAMB08), and Baynesfield, December 2008 (BF09). The F_1 hybrids and standard hybrid checks were laid out in the field in two replications using a 9 x 6 alpha lattice design in each environment. Inbred parents were planted in a 14 x 2 randomised complete block design (RCBD) with two replications on the same day as the hybrids. The plot size for both the hybrids and parental lines in each environment was two rows, 3 m long, with 0.75 m inter-row spacing and 0.3 m intra-row spacing, except for Mpongwe where plots were one row, 5 m long with 0.75 m between rows and 0.3 m between the plants. Plant population densities were about 44 000 per hectare at all the locations.

A susceptible maize hybrid and inbred line (N3) were used as borders for the hybrid and inbred trials, respectively. Fertiliser was applied at the rate of 120 kg N, 33 kg P, and 44 kg K. Standard cultural practices including ploughing and disking, hand planting, hand weeding and/or application of herbicides and fertilizers were followed at each site.

Grey leaf spot (GLS) disease severity was assessed twice at mid-silking (GLS1) and at hard dough stages (GLS2), based on visual assessment of the whole plot using a 1-9 logarithmic rating scale, where 1 = 0% , 2 = <1%, 3 = 1-3%, 4 = 4-6%, 5 = 7-12%, 6 = 13-25%, 7 = 26-50%, 8 = 51-75% and 9 = 75-100% leaf area showing disease symptoms. The scores were further classified into the following disease reaction types; 1.0 = symptomless, 2.0-4.0 = resistant, 4.1-5.0 = moderately resistant, 5.1-6.0 = moderately susceptible, 6.1-9.0 = susceptible. The score recorded at the hard-dough stage was used for statistical analysis. Heterosis for GLS disease scores for each hybrid was estimated for the three environments that included parents, using mid-parent (MP) scores (Falconer and Mackay, 1996) according to the following equation:

MPH (%) = $100*(F_1\text{-MP})/MP$,

Where F_1 = mean of the F_1 hybrid performance, MP = mean of the two parents making the cross.

6.2.4 Field evaluation of the different generations

The different generations were evaluated at Cedara Agricultural Research Station in South Africa. Trials were hand-planted on 27 November 2008. Each cross (R x S) was considered to be a separate experiment, but the general procedures applied were the same for all the crosses. The experiment was laid out as a randomized complete block design with two replications. For each cross, there were 2 rows of each of the P_1, P_2, F_1 and F'_1 generations, 8 rows each of the F_2 and F'_2 generations, and 4 rows of each of the BCP_1 and BCP_2 and their reciprocal generations. Rows were 2.8 m long with 0.75 m inter-row spacing and 0.2 m intra-row spacing. Plots were thinned to 15 plants per row. The final number of plants was variable for each generation. A susceptible inbred line was used as a spreader and planted at the borders of each block. Standard cultural practices including ploughing and disking, hand planting, hand weeding and/or application of herbicides and fertilizers were practiced each season. Disease severity was rated by visually estimating the percent leaf area blighted (necrotic) on individual plants following a 1-9 logarithmic increment rating scale described for the diallel evaluation, at the hard dough developmental stage of maize.

6.2.5 Data analysis for diallel analysis

The GLS severity score assessed at hard-dough stage (GLS2) was used for analysis. The F_1 hybrids and the inbred parents were treated as fixed effects in the statistical analysis and environments (both spatial and temporal environments) as random effects. The researcher had no control over the environment. This implied that interest was in selecting inbreds that would perform well on an average site through the years. Data were analysed for combining ability using the Diallel SAS05 program in SAS (Zhang *et al*., 2005). Only the 45 F_1 experimental hybrids were used in the analysis for combining abilty effects. To estimate the general combining ability (GCA) and specific combining ability (SCA) effects; Griffing's diallel analyses, Model 1 (fixed genotype effects), Method 4 (crosses only) was used according to the model:

$Yijk = \mu + gi + gj + sij + eijk$

Where*: Yijk = observed measurement for the ijth cross in the kth replication/ environment combination, μ = overall mean, gi and gj = GCA effects for the ith and jth parents respectively, sij = SCA effect for the ijth cross, eijk = error term associated with the ijth cross evaluated in the kth replication/environment combination.*

The interaction terms were used to test for the significance of the corresponding main effects (Zhang and Kang, 1997). The environments and replications within environments were considered random and therefore tested against the residual error term.

6.2.6 Generation mean analysis

Mean disease ratings and variance of generations within each replication were calculated from individual plant ratings in the P_1, P_2, F_1, F'_1, F_2, F'_2, $BCP_1(F_1)$, $BCP_1(F'_1)$, $BCP_2(F_1)$ and $BCP_2(F'_1)$ generations using Genstat 12 (Payne *et al*., 2009). Frequency distribution curves of the F_2 and backcross generations derived from the crosses were plotted using the data analysis programme in Microsoft Excel 2007. Data for each cross were analysed using PROC GLM procedure in SAS 9.1 (SAS Institute, 2002). Where significant differences between generations were observed, separation of means was carried out with the t- test ($P \leq 0.05$) and this was also used to test for differences in the F_1 reciprocal generations. The ten generations for each cross were used to estimate the genetic effects using the following model (Kang, 1994):

$Y = m + \acute{\alpha}a + \beta d + \acute{\alpha}^2 aa + 2\acute{\alpha}\beta ad + \beta^2 dd$

Where*: m = mid-point value, ά and β are the matrix coefficients for the generations, a = cumulative additive effect, d = cumulative dominance effect, aa = cumulative additive x additive effect, ad = cumulative additive x dominance effect, dd = cumulative dominance x dominance effect*.

The GMA model was analysed in SAS following procedures described by Kang (1994).

6.2.7 Heritability estimates

Since individual plants were scored, variances among the plants within each generation were used to estimate generation variances. Broad-sense heritability estimates were estimated using the equation:

$H^2 = 100*(\sigma^2_g / \sigma^2 F_2)$

Where: σ^2_g = genetic variance, $\sigma^2 F_2$ = variance of F_2 generation.

The estimate of $\sigma^2 g = \sigma^2 F_2 - \sigma^2_e$, and σ^2_e = environmental variance = $(nP_1 s^2 P_1 + nP_2 s^2 P_2 + nF_1 s^2 F_1)/ (Ne)$ (Wright, 1968); where n = the number of plants in each generation, $s^2 P_1$, $s^2 P_2$ and $s^2 F_1$ = variances of the Parent 1 (P_1), Parent 2 (P_2) and F_1 generations and Ne = $nP_1 + nP_2 + nF_1$.

Narrow-sense (h^2) heritability estimates were calculated using the equation:

$h^2 = 100*[2 \sigma^2 F_2 – (\sigma^2 BCP_1 + \sigma^2 BCP_2)]/ \sigma^2 F_2$ (Warner, 1952).

6.2.8 Minimum number of genes (effective factors)

The minimum number of genes controlling resistance to GLS in each cross was estimated using the formula (Wright, 1968):

$N = (X_1 – X_2)^2/8*(\sigma^2 F_2 - \sigma^2_e)$, where $\sigma^2 F_2 - \sigma^2_e = \sigma^2_g$

Where N = number of genes, X_1 = mean resistance of parent 1, X_2 = mean resistance of parent 2, $\sigma^2 F_2$ = variance of F_2 generation, σ^2_e = environmental variance within family.

The assumptions being that all the genes controlling the trait are unlinked; they affect the trait equally in size and direction; and there are no dominance or epistasis effects involved.

6.2.9 Heterosis

Mid-parent heterosis (MPH) was calculated as the performance of F_1 or (F'_1) compared with the average performance of its parents (Fehr, 1991). The following formula was used:

$MPH = 100*([M_{F1}-(M_{P1} + M_{P2})/2]/(M_{P1}+M_{P2})/2)$,

Where: M_{F1}, M_{P1} and M_{P2} are the mean disease severity scores for the parents (P_1 and P_2) and F_1 (or F'_1) generations, respectively.

6.2.10 Average degree of dominance

Average degree of dominance (ADD) was determined using the method of Mather and Jinks (1982), where:

ADD = $[M_{F1}-(M_{P1} + M_{P2})/2]/ (M_{P1}-M_{P2})/2$, where;

M_{P1}, M_{P2} and M_{F1} are the mean disease severity ratings in the parental and F_1 (or F'_1) generations, respectively.

6.3 Results

The results of the contrast of GLS severity scores for the experimental hybrids versus checks are presented in Table 6.2. The entries (experimental hybrids plus checks), experimental hybrids, hybrid checks and their interactions with the environments were all highly significant (P<0.001). The contrast of the GLS severity scores for the experimental hybrids against the checks was highly significant (P<0.001), but the interaction with the environment was not significant (P>0.05). The means for the GLS severity scores of the experimental hybrids and checks were 2.6 and 3.3, respectively.

Table 6.2. Analysis of variance for GLS severity scores: Experimental hybrids versus check hybrids.

Source	DF	SS	Mean Square
Environment (Env)	**5**	**269.31**	**53.86*** **
Rep(Env)	**6**	**5.031**	**0.84^{NS}**
Entry	**53**	**1650.53**	**31.14*** **
Experimental hybrids (Exp)	44	1301.33	29.58***
Hybrid Checks (Chks)	8	305.97	38.25***
Exp vs chks	1	45.82	45.82***
Env*Entry	**264**	**580.19**	**2.20*** **
Env*Exp	220	451.43	2.05***
Env*Chks	39	92.47	2.37***
Env*Exp vs Chks	5	33.70	6.74^{NS}
Error	**317**	**155.47**	**0.49**
Corrected Total	**645**	**2660.53**	
Means			
Experimental hybrids	2.6		
Checks	3.3		

*, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001, respectively, NS = non-significant (P>0.05).

6.3.1 Reaction of the inbreds and hybrids to GLS disease infection

The GLS disease pressure was different in the six environments as depicted by the different maximum scores recorded for both the inbreds and the F_1 hybrids (Table 6.3 and 6.4). The ten inbred lines (Table 6.3) used as parents in this study showed significant differences (P≤0.05) in their reaction to GLS infection. Means of the parents in two of the environments (C09 and BF09) varied from 1.0 to 9.0. Inbred lines that were consistently symptomless to resistant (scores 1.0 - 4.0) based on the classification used in this study included; A1220-4, CZL00009, CZL00001, CML205 and CML443. The most susceptible parent was N3 with scores ranging from 8.5 - 9.0. The other inbreds had variable reactions at the two sites and these were CML445, A16, MP18 and CML488, were moderately susceptible to susceptible at Baynesfield (BF09) location compared to Cedara (C09).

There was significant variation (P≤0.05) for GLS scores amongst the F_1 hybrids (Table 6.4). The number of hybrids that had GLS scores below 2.0 ranged from 17 (BF09) to 26 (C208). Most of the hybrids exhibited a resistant reaction (scores 2.0 - 4.0) in all the six environments. Baynesfield location recorded the highest score of 9.0 for GLS severity, whereas at Rattray Arnold Research Station (RARS08) and Cedara, January 2008 planting (C208), the highest scores were only 6.0 and 5.0 respectively.

Table 6.3 Reactions of the ten inbred parents tested over two environments in 2008/9 season

Entry	Line	C09†	BF09	Across environments	Disease Reaction‡
35	N3	8.5	9.0	8.8	S
46	A16	6.5	6.0	6.3	S
1	CML445	3.5	6.5	5.0	MS
55	CML488	3.5	6.0	4.8	MR
50	MP18	2.5	7.5	5.0	MS
53	CML443	2.0	1.0	1.5	R
41	CML205	2.0	2.0	2.0	R
28	CZL00001	1.5	1.0	1.3	R
11	A1220-4	1.5	2.5	2.0	R
20	CZL00009	1.0	1.0	1.0	R
Mean		3.3	4.3	3.8	
LSD(0.05)		1.4	1.7	1.0	

† C09 = Cedara November 2008 planting, BF09 = Baynesfield December 2008 planting. ‡Disease reactions: R = resistant, MR = moderately resistant, S = susceptible and MS = moderately susceptible.

Table 6.4 Reactions of the 45 F_1 hybrids tested over six environments between 2007 and 2009.

Entry	C108†	C09	BF09	RARS08	ZAMB08	C208	Across environments	Disease Reaction‡
20 most resistant hybrids								
CML445xCZL00009	1.0	1.0	1.0	1.0	1.5	1.0	1.1	R
A1220-4xCZL00001	1.0	1.5	1.0	1.0	1.0	1.0	1.1	R
CZL00009xCZL00001	1.5	1.0	1.0	1.5	1.0	1.0	1.2	R
CZL00001xA16	1.5	1.0	1.5	1.0	1.0	1.0	1.2	R
CML205xMP18	1.0	1.5	1.0	1.0	1.0	1.5	1.2	R
CZL00009xCML205	1.5	1.5	1.5	1.0	1.0	1.0	1.3	R
CZL00009xA16	1.0	1.0	1.0	2.5	1.0	1.0	1.3	R
CZL00009xMP18	1.0	1.0	1.0	2.0	1.0	1.5	1.3	R
N3xCML205	1.0	1.0	2.0	1.0	1.0	1.5	1.3	R
CZL00009xCML443	2.0	1.5	1.0	1.5	1.0	1.0	1.3	R
CZL00001xMP18	1.0	1.0	1.0	2.0	1.5	1.5	1.3	R
A1220-4xCML205	1.0	2.0	3.0	1.0	1.5	1.0	1.6	R
CZL00001xCML205	1.0	1.5	2.0	2.0	2.0	1.0	1.6	R
A1220-4xCML443	3.0	1.5	1.0	1.0	1.0	2.5	1.7	R
A16xCML443	1.5	1.0	2.5	1.0	1.5	2.5	1.7	R
CZL00009xN3	3.0	1.5	2.5	1.0	1.0	1.5	1.8	R
CZL00001xCML488	1.5	2.0	3.0	1.0	2.0	1.5	1.8	R
CZL00009xCML488	1.5	1.5	3.0	2.5	1.5	2.5	2.1	R
CZL00001xN3	2.0	2.0	4.5	1.0	1.5	1.5	2.1	R
A1220-4xMP18	2.0	2.5	3.0	2.0	2.0	2.0	2.3	R
Resistant checks								
P27	1.0	2.0	1.5	1.0	1.0	1.0	1.3	R
S51	1.5	1.5	1.5	2.0	1.5	1.0	1.5	R
S71	2.0	2.5	3.5	1.5	1.5	1.5	2.1	R
P77	1.0	3.0	4.5	2.0	1.5	1.5	2.3	R
5 most susceptible hybrids								
N3xMP18	8.0	2.0	6.0	4.5	6.0	3.5	5.0	MS
A1220-4xN3	5.5	7.0	8.5	4.0	5.5	3.0	5.6	MS
N3xCML488	5.0	6.0	8.0	3.0	7.0	5.0	5.7	MS
CML445xN3	8.0	8.0	8.0	4.0	5.5	3.5	6.2	S
N3xA16	8.0	8.0	8.5	6.0	7.0	5.0	7.1	S
Susceptible checks								
P57	6.5	5.5	8.5	4.0	3.8	2.5	5.1	MS
P067	6.0	6.0	8.0	4.0	3.0	6.0	5.5	S
N72	3.0	7.5	8.0	5.5	4.5	6.5	5.8	MS
Mean	2.9	2.7	4.0	2.2	2.4	2.1	2.6	
LSD(0.05)	1.3	1.4	1.2	1.7	1.3	1.3	0.5	

†C108 = Cedara November 2007 planting, C208= Cedara January 2008 planting, C09 = Cedara November 2008 planting, BF09 = Baynesfield December 2008 planting, RARS08 = Rattray Arnold Research station December 2007 planting and ZAMB08 = Mpongwe, Zambia January 2008 planting. ‡Disease reactions: R = resistant, MR = moderately resistant, S = susceptible and MS = moderately susceptible.

6.3.2 Combining ability estimates

Combined analysis across environments showed highly significant (P≤0.001) Environment, Entry, GCA and SCA main effects and all the interactions (Table 6.5). The GCA effects were 2.5 times larger than the SCA effects. When the entry (hybrid) sum of squares was partitioned, the GCA effects accounted for 71% and the SCA about 28% of the variation among the hybrid GLS scores.

Table 6.5 Analysis of variance for grey leaf spot disease scores of 45 F_1 hybrid crosses tested over six environments between 2007 and 2009 and the contribution of the different genetic effects to the total entry sum of squares.

Source	df	Type I SS	Mean Square
Environment (ENV)	5	183.65	36.73***
REP(ENV)	6	2.28	0.38
Entry	44	1301.33	29.58***
GCA	9	930.48	103.39***
SCA	35	370.84	10.60***
GCA contribution (%)		**71.50**	
SCA contribution (%)		**28.49**	
ENV*Entry	220	451.43	2.05***
GCA*ENV	45	221.84	4.93***
SCA*ENV	175	229.59	1.31***
Pooled error	264	118.22	0.45

*, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001, respectively.

The GCA effects of the ten parents are presented in Table 6.6. The GCA effects were highly significant (P≤0.001) for CZL00009, CZL00001, N3 and A16 at all the six sites, for CML205 and CML443 at five of the sites, CML445 and CML488 at four sites, and A1220-4 and MP18 at three sites. For disease resistance, negative GCA and SCA effects are desirable. The resistant parents; CZL00009, CZL00001, CML205 and CML443 had negative GCA effects across environments. Positive GCA effects across environments for the disease scores were observed for CML445, N3, A16 and CML488. Parents A1220-4 and MP18 had some of the effects negative and some positive, depending on the environment, but generally they had non-significant (P>0.05) GCA effects.

Table 6.6. Estimate of general combining ability (GCA)† effects for Grey leaf spot disease scores evaluated in six environments between 2007 and 2009

Parent	GLS Mean Score	Environment						GCA across environments
		C108	C09	BF09	RARS08	ZAMB08	C208	
CML445	4.2	1.03***	0.87***	0.89***	0.35	0.43**	-0.08	0.58***
A1220-4	1.7	-0.23	0.49***	-0.30*	-0.28	-0.39**	0.05	-0.11
CZL00009	1.0	-1.54***	-1.45***	-2.55***	-0.59**	-1.45***	-0.76***	-1.39***
CZL00001	1.2	-1.79***	-1.26***	-2.18***	-0.90***	-1.20***	-0.89***	-1.37***
N3	7.8	2.59***	2.30***	2.70***	0.98***	2.11***	1.05***	1.95***
CML205	1.8	-1.10***	-0.26	-0.80***	-0.46*	-0.51***	-0.64***	-0.63***
A16	4.7	0.40**	0.68***	1.20***	0.98***	1.05***	0.80***	0.85***
MP18	3.7	0.28	-0.83***	-0.49***	0.73***	0.24	0.11	0.01
CML443	1.3	0.28	-0.89***	-0.43***	-0.90***	-0.70***	-0.33*	-0.49***
CML488	3.7	0.09	0.36*	1.95***	0.10	0.46**	0.68***	0.60***

†Negative GCA effects were desirable. *, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001

The specific combining ability estimates for the 45 F_1 hybrids are shown in Table 6.7. The effects were variable in the six environments with C208 having only eight hybrids with significant (P≤0.05) SCA effects. For the other five environments, the number of hybrids with significant positive or negative SCA effects ranged between 13 and 24. The SCA effects were generally low at C208 compared to the other environments. Eight hybrids; CML445 x CZL00001, CML445 x CZL00009, CZL00009 x N3, CZL00001 x N3, CZL00001 x A16, N3 x CML205, CML205 x MP18 and A16 x CML443 had significant (P≤0.05) negative SCA effects in at least three environments. Of these eight hybrids, N3 x CML205, N3 x CZL00009, CZL00001 x N3 and A16 x CML443 had high negative values across the environments. Hybrids CML445 x A1220-4, CML445 x CML488, A1220-4 x A16, A1220-4 x MP18, A1220-4 x CML443, N3 x MP18, N3 x CML443, CML205 x CML443, MP18 x CML488 and CML443 x CML488 had significant (P≤ 0.05) negative values in at least one environment. The remainder of the hybrids had either significant positive (P≤0.05) or non-significant (P≥0.05) effects. On the whole, the hybrids N3 x A16 and CML205 x A16 had high positive significant (P≤0.01) SCA effects.

Table 6.7 Hybrids with significant mean estimates of specific combining ability (SCA)† effects for GLS disease severity scores at the six environments for the set of diallel crosses among ten maize inbred lines.

Cross	GLS Mean Score	Environment‡						SCA Across environments
		C108	C208	C09	BF09	RARS08	ZAMB08	
CML445x A1220-4	2.58	-0.67	-0.43	0.14	-0.30	-1.15*	-0.44	-0.47
CML445xCZL00009	1.08	-1.35***	-0.12	-0.92*	-1.05***	-0.84	0.13	-0.69*
CML445xCZL00001	1.00	-1.10***	0.01	-1.11**	-1.42***	-0.53	-0.63	-0.80**
CML445xN3	6.17	1.52***	0.57	2.33***	0.70*	0.60	0.56	1.05***
CML445xCML205	3.25	0.71	0.26	1.39***	0.20	1.53**	0.19	0.71*
CML445xA16	4.25	-0.29	-0.18	-0.55	0.70*	0.60	1.13**	0.23
CML445xMP18	3.33	0.83*	0.01	-0.55	0.39	0.35	-0.06	0.16
CML445xCML443	2.58	1.33***	-0.56	-0.49	0.33	-0.53	-0.63	-0.09
CML445xCML488	3.67	-0.98**	0.44	-0.24	0.45	-0.03	-0.25	-0.10
A1220-4xN3	5.58	0.27	-0.06	1.70***	2.39***	1.22*	1.38***	1.15***
A1220-4xA16	3.50	0.46	1.19***	0.33	-0.11	0.22	-1.06**	0.17
A1220-4xMP18	2.25	-0.92*	-0.12	0.33	0.08	-0.53	-0.25	-0.23
A1220-4xCML443	1.67	0.08	0.82*	-0.61	-1.99***	0.10	-0.31	-0.32
A1220-4xCML488	3.17	1.27***	-0.68	-0.36	-0.36	0.60	0.06	0.09
CZL00009xCZL00001	1.17	1.96***	0.69	1.20**	2.01***	0.91	1.25***	1.34***
CZL00009xN3	1.75	-0.92*	-0.74	-1.86***	-1.36***	-1.47**	-2.06***	-1.40***
CZL00009xCML205	1.25	1.27***	0.44	0.70	1.14***	-0.03	0.56	0.68*
CZL00009xA16	1.25	-0.73	-0.99*	-0.74	-1.36***	0.03	-1.00*	-0.80**
CZL00009xCML443	1.33	0.40	0.13	1.33**	0.26	0.91	0.75	0.63*
CZL00001xN3	2.08	-1.67***	-0.62	-1.55***	0.26	-1.15*	-1.81***	-1.09***
CZL00001xCML205	1.58	1.02**	0.57	0.51	1.26***	1.28**	1.31***	0.99***
CZL00001xA16	1.17	0.02	-0.87*	-0.92*	-1.24***	-1.15*	-1.25***	-0.90***
CZL00001xCML488	1.92	0.33	-0.24	0.89*	-0.49	-0.28	0.38	0.10
N3xCML205	1.33	-3.35***	-0.87*	-3.05***	-3.61***	-1.59**	-3.00***	-2.58***
N3xA16	7.08	2.15***	1.19**	2.51***	0.89**	1.97***	1.44***	1.69***
N3xMP18	5.00	2.27***	0.38	-1.99***	0.08	0.72	1.25***	0.45
N3xCML443	4.25	0.27	-1.18**	1.08**	1.01**	-0.15	0.19	0.20
N3xCML488	5.67	-0.54	1.32***	0.83*	-0.36	-0.15	2.06***	0.53
CML205xA16	4.75	3.33***	0.38	1.08**	2.89***	0.91	3.06***	1.94***
CML205xMP18	1.17	-1.04**	0.07	0.08	-1.42***	-1.34**	-1.13**	-0.80**
CML205xCML443	1.00	-1.04**	0.01	-0.36	-1.49***	0.28	-0.19	-0.46
A16xMP18	3.33	-1.04**	0.13	0.14	1.08**	-0.28	-0.69	-0.11
A16xCML443	1.67	-2.04**	0.07	-1.30***	-1.99***	-1.15*	-1.25***	-1.28***
A162xCML488	3.08	-1.85***	-0.93	-0.55	-0.86**	-1.15*	-0.38	-0.95***
MP18xCML443	2.67	0.08	-0.24	0.70	1.20***	0.10	1.56***	0.57
MP18xCML488	3.00	0.77*	-0.74	-0.05	-1.67***	1.10*	-0.56	-0.19
CML443xCML488	3.17	1.27***	0.69	-0.99*	2.76***	-0.28	-0.63	0.47

†Negative SCA effects desirable for GLS resistance. *, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001. ‡C108 = Cedara November 2007 planting, C208 -= Cedara January 2008 planting, C09 = Cedara November 2008 planting, BF09 = Baynesfield December 2008 planting, RARS08 = Rattray Arnold Research station December 2007 planting and ZAMB08 = Mpongwe, Zambia January 2008 planting

6.3.3 Estimates of heterosis

Mid-parent heterosis values were variable among the hybrids (Fig. 6.1). Fifty-one per cent (23 out of 45) of the hybrids had negative heterosis values varying from -5% to -165.83%. Most of the crosses involving CZL00009, CZL00001, MP18, CML443 and CML488 had negative heterosis. These same hybrids also had significant negative SCA effects (Table 6.7).

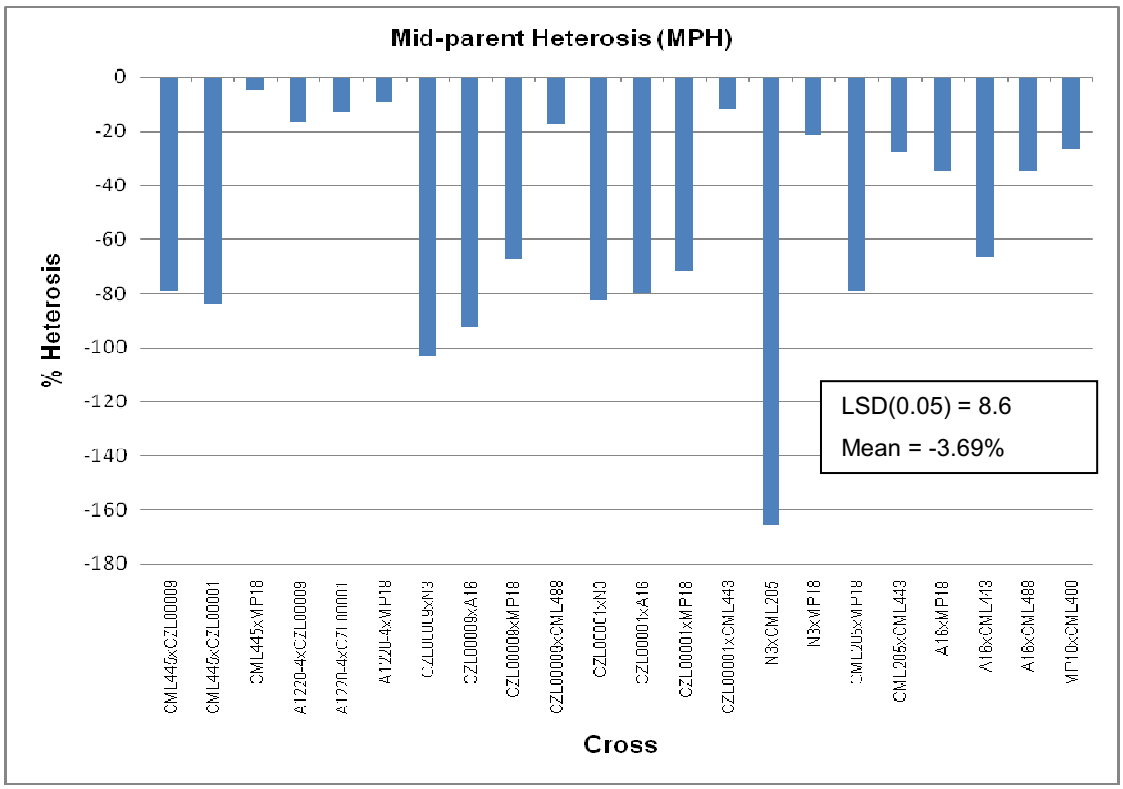

Figure 6.1 Percentage negative mid-parent heterosis of the 45 F_1 maize hybrids involving ten inbred lines with different levels of resistance to GLS evaluated in three environments between 2007 and 2009.

6.4 Generation mean analysis

6.4.1 Disease development and frequency distributions

The total number of plants used to calculate the means of the generations and the mean disease scores for the three crosses are shown in Table 6.8. Mean ratings for the susceptible parents N3 and B17 ranged between 6.5 and 8.3 in the different populations they were involved in. The resistant parents; A1220-4, B17 and A15 all had mean scores ranging between 1.4 and 2.2. All the other generations for each of the three crosses had disease scores lower than the susceptible parent (Table 6.9).

Table 6.8 Total numbers of plants and mean disease ratings of generations based on individual plant ratings from the three crosses evaluated for GLS severity at Cedara

	CML441 (R) x N3 (S)		A1220-4 (R) x B17 (S)		A15 (R) x B17 (S)	
Generation‡	Number of plants	Mean† disease score ± se	Number of plants	Mean disease score ± se	Number of plants	Mean disease score ± se
P1	46	2.2±0.13	29	1.4±0.11	56	2.1±0.09
P2	44	8.3±0.15	55	6.5±0.17	49	7.6±0.12
F1	37	4.9±0.25	57	3.8±0.17	64	3.7±0.18
F'1	48	4.7±0.19	60	3.1±0.15	73	3.7±0.19
F2	146	4.6±0.15	207	3.5±0.10	190	4.5±0.14
F'2	135	3.8±0.17	202	3.4±0.12	184	4.1±0.15
BCP1(F1)	84	3.2±0.14	103	2.8±0.10	84	3.7±0.22
BCP1(F'1)	96	2.7±0.14	86	3.2±0.17	122	2.5±0.09
BCP2 (F1)	83	6.7±0.22	100	4.4±0.15	113	5.7±0.18
BCP2(F'1)	92	6.9±0.17	105	4.6±0.19	96	6.0±0.18

†Means obtained from the total number of individual plants scored for GLS severity. ‡**P_1** is the parent appearing first in each cross and **P_2** is parent appearing second in each cross, F1 = (P_1 x P_2) F'$_1$ = (P_2 x P_1); F'$_2$, BCP$_1$ x F'$_1$ and BCP$_2$ x F'$_1$ are the reciprocal generations of F_1, F_2, BCP$_1$ x F_1 and BCP$_2$ x F_1 respectively. The resistance level of each parent is indicated by an R or S. where R = resistant and S = susceptible.

For all the three crosses, means of both the F_2 and F'$_2$ generations were not significantly different (P≤0.05) from the F_1 and F'$_1$ generation (Table 6.9). In general, the F_1 and F'$_1$ for all the three crosses had a rating lower than the mean of the two parents, with the exception of the F_1 generation for cross CML441 x N3, which had a higher disease severity rating. There were no significant differences between the F_1 reciprocal generations for all the three crosses. The mean disease scores of the backcross generations to F_1 or F'$_1$ crossed to either of the parents appeared closer to the recurrent parent for all the crosses. Frequency distributions of the F_2 and backcross progeny scores showed continuous variation (Fig. 6.2). The distributions varied from normal to slightly skewed either towards resistance or susceptibility depending on the cross.

Table 6.9: Generation means for GLS ratings of the R x S crosses

CML441 (R) x N3 (S)		A1220-4 (R) x B17 (S)		A15 (R) x B17 (S)	
Generation	Mean	Generation	Mean	Generation	Mean
P2	8.3 a	P2	6.8 a	P2	7.5 a
BCP2(F1)	7.9 ab	BCP2(F'1	4.7 b	BCP2(F'1	6.0 b
BCP2(F'1	7.8 ab	BCP2(F1)	4.6 b	BCP2(F1)	5.7 b
F1	6.4 bc	F1	4.1 b	F2	4.5 c
F'1	5.5 cd	F2	3.7 b	F'2	4.1 cd
F2	5.3 cde	F'2	3.5 bc	BCP1(F1)	3.7 d
F'2	4.5 cdef	BCP1(F'1	3.5 bc	F'1	3.7 d
BCP1(F1)	4.0 def	F'1	3.4 bc	F1	3.7 d
BCP1(F'1	3.5 ef	BCP1(F1)	3.1 bc	BCP1(F'1	2.6 e
P1	3.0 f	P1	1.9 c	P1	2.1 e
Mean of the parents	**6.0**	**Mean of the parents**	**4.4**	**Mean of the parents**	**4.8**
LSD(0.05)	**0.6**		**0.5**		**0.5**

Means followed by the same letter in a column for each cross are not significantly different at P=0.05. R and S = resistant and susceptible, respectively

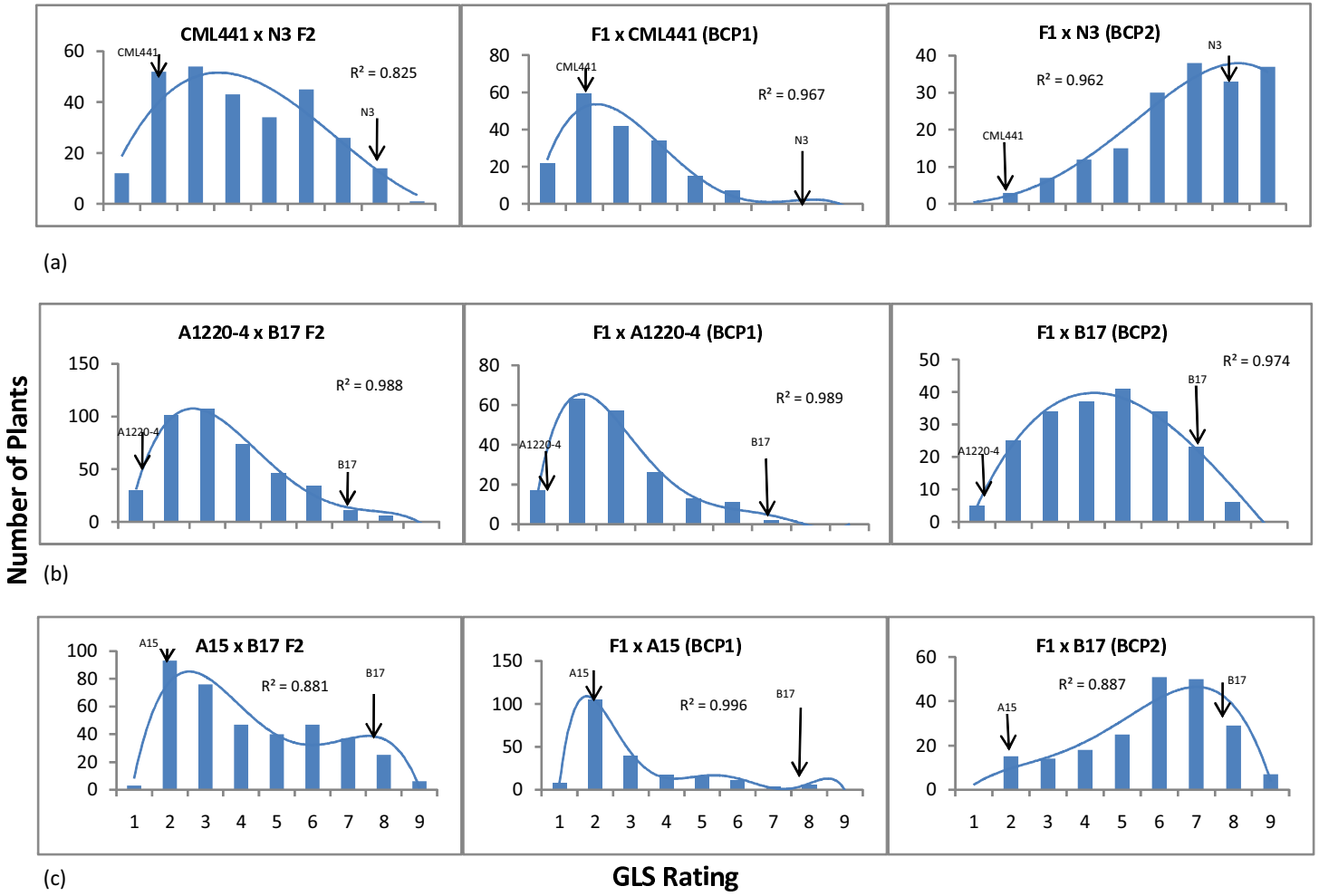

Figure 6.2 Frequency distributions of Grey leaf spot (GLS) ratings of individual plants of maize F_2 and backcross generations from the three R x S crosses. Arrows indicate the position of the two parents involved in the cross.

6.4.2 Genetic effects

Analysis of variance showed a highly significant (P≤0.001) generation effect for all the three crosses (Table 6.10). Additive effects were highly significant (P≤0.001) for all the crosses. Significant (P≤0.001) dominance effects were observed only for the cross A15 x B17. For CML441 x N3 cross, additive x additive effects were significant (P≤0.05) and additive x dominance effects were significant for the A1220-4 x B17 cross.

Table 6.10: Analysis of variance on the generation mean scores of three maize crosses evaluated for GLS severity during at Cedara

Source of variation	df	CML441 (R) x N3(S)	A1220-4 (R) × B17 (S)	A15 (R) × B17(S)
		Mean square	**Mean Square**	**Mean square**
Replication	1	1.02	0.36	0.06
Generations	9	8.47***	3.38***	5.36***
a	1	69.85***	26.85***	43.75***
d	1	0.07	0.68	2.67***
aa	1	2.94*	0.43	0.02
ad	1	1.37	1.73*	0.00
dd	1	0.44	0.10	0.25
Error	9	0.66	0.60	0.11
CV		14.10	17.29	7.45

*, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001, respectively. R and S = resistant, and susceptible, respectively

The estimates of the genetic effects are presented in Table 6.11. The significant additive effects for all the three crosses were negative. The additive effects contributed between 89% and 93% to the total sum of squares (SSq) for the generations. The estimate for dominance effects for A15 x B17 cross was also negative and contributed 7% to the total sum of squares of the generations. Estimates for the non-allelic interactions for CML441 x N3 and A1220-4 x B17 crosses, were all positive and contributed 5% and 7%, respectively, to the generation sum of squares.

Table 6.11. Estimates of genetic effects ± se and the relative contributions of the genetic effects to the model total sum of squares (SSq) in brackets of the generation means in the different crosses

Model	CML441 (R) × N3 (S)	A1220-4 (R) × B17 (S)	A15 (R) × B17 (S)
m	3.7 ± 0.9***	4.0 ± 0.5***	4.8 ± 0.4***
a	-3.1 ± 0.4*** **(92.0%)**	-2.5 ± 0.3*** **(89.3%)**	-2.8 ± 0.2*** **(92.7%)**
d	NS	NS	-1.2 ± 0.3*** **(7.2%)**
aa	2.0 ± 0.9* **(5.4%)**	NS	NS
ad	NS	2.6 ± 1.1* **(7.1%)**	NS
dd	NS	NS	NS
R^2 for the Model	0.9	0.8	0.9
CV (%)	6.2	9.1	5.9

*, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001 respectively. NS = non-significant. R and S = Resistant, and susceptible, respectively

6.4.3 Heritability estimates, minimum number of genes controlling GLS resistance, heterosis and average degree of dominance

Broad-sense heritability estimates for the crosses ranged from 64% to 92% (Table 6.12), whereas narrow-sense heritability estimates were between 54% and 77%. The estimated minimum number of genes controlling GLS resistance ranged from two to three. Mid-parent heterosis ranged from -0.6 to -6.0 % (Table 6.12). The average degree for dominance (ADD) for resistance in the crosses varied from 0.0 to 0.1.

Table 6.2 Estimates of broad-sense heritability (H^2), narrow-sense heritability (h^2), minimum number of genes (MNG), mid-parent heterosis and average degree of dominance (ADD) of resistance to grey leaf spot in maize

Cross	**H^2 (%)**	**h^2 (%)**	**MNG**	**Mid-parent heterosis (%)**		**ADD**	
				F_1	F'_1	F_1	F'_1
CML441 (R) x N3 (S)	76.7	74.2	1.8	-1.2	-2.5	0.0	0.0
A1220-4 (R) x B17 (S)	64.1	54.4	2.6	-5.9	-5.4	0.0	0.1
A15 (R) x B17 (S)	92.0	77.0	1.5	-6.1	-5.9	0.1	0.1

6.5 Discussion

6.5.1 Diallel analysis

6.5.1.1 Disease development

Disease development was variable in the six environments. Baynesfield (BF09) had the highest disease pressure, followed by Cedara (C09) and C108, with C208 and RARS08 having the lowest disease levels. The normal seasons' plantings at Cedara (C108 and C09), that is the November 2007 and 2008 plantings had relatively similar disease pressures. The January 2008 planting at Cedara was a late season planting and had less disease pressure. This could have been a result of mid-season drought that was experienced from mid January 2008 to end of March 2008 resulting in unfavourable conditions for GLS disease development. Most of the fields at Cedara and Baynesfield locations were under reduced tillage suggesting high inoculum levels in the plant debris and soil, which could have contributed to the high disease levels in the normal season. At Rattray Arnold Research Station, Zimbabwe and Mpongwe, Zambia, deep ploughing is practiced, which could lead to a reduction in inoculum at the beginning of the season.

There was a wide range of severity levels among the inbred parents used in the diallel cross. The most susceptible was N3 and the most resistant were A1220-4, CZL00009, CZL00001, CML205 and CML443. However, some of the inbred parents behaved differently depending on the environment. Inbreds CML445, CML488 and MP18 appeared resistant in the two Cedara environments (C108 and C09) but moderately susceptible at Baynesfield (BF09). Baynesfield location had the highest percentage of inbreds and hybrids with high disease severity ratings implying very high inoculum levels compared to C108 and C09 environments. It appears disease pressure influenced their reaction to GLS infection. It means, therefore, that for good discrimination of the inbreds or hybrids, either artificial inoculation or "hot spot areas" of the disease should be employed.

There was significant differentiation amongst the hybrids in at least four of the environments (BF09, C108, C09 and ZAMB08). Frequency distributions for the environments indicated skewness towards resistance. Fifty-seven per cent of the hybrids at the sites with relatively high disease pressure were in the resistant category. These resistant hybrids involved crosses with parents CZL00009, CZL00001, A1220-4 and CML443. The four inbred lines can, therefore, be additional sources of resistance to GLS in breeding programmes. Inbred line A1220-4 was derived in part from ZM605, CML395 and CML444 (see Table 6.1), which form the basis of the most productive hybrids in the

medium to high altitude environments in tropical east and southern Africa. The most susceptible hybrids had two common parents; N3 and A16, while CML445 and CML488 contributed to susceptibility in some, but not all of the hybrids they were involved. Historically, lines such as CML312, CML395, and CML444 have been amongst CIMMYT's most successful lines, while CML488 is amongst the most promising CIMMYT lines (CIMMYT, 2001). Therefore, these susceptible lines require improvement for GLS resistance.

6.5.1.2 Combining Ability Estimates

The significant GCA and SCA main effects indicated that both additive and non-additive gene effects were important in the resistance to GLS in the maize inbred lines used. The GCA effects were 2.5 times larger than the SCA effects implying the predominance of additive over non-additive gene action. In this study GCA effects contributed more than 70% and SCA about 28% of the entry (hybrid) sum of squares. Studies by other researchers using other populations have also shown the predominance of additive over non-additive gene action. Vivek *et al*. (2009) reported that GCA accounted for 77% of the variation for GLS resistance. Derera *et al*. 2008 reported an 88% GCA contribution, whilst studies by Thompson *et al*. (1987) and Ulrich *et al*. (1990) reported 100% GCA contribution to the variation for GLS resistance. These results apply to the reference population used and the variations observed amongst different researchers are a result of the different lines used and the environment.

The significant environment x GCA and environment x SCA interaction were mainly as a result of differences in disease pressure in the different environments. In general, the ranking of the inbreds or hybrids did not change, except for parents CML445, CML488 and MP18, which were resistant in two of the sites and susceptible in one site. This type of interaction which does not involve changes in ranking does not pose any serious problems as breeding for specific adaption is not required. Derera *et al*. (2008) and Lipps *et al*. (1998) in independent studies reported similar interactions where hybrid ranking remained the same and only disease severity at the different locations and years contributed to the interactions. This means, therefore, that one can select for GLS resistance at one reliable site and still be able to deploy the resistant lines or hybrids to other environments in which they are adapted. In this study, the two environments which were promising included Baynesfield and Cedara.

Parents A1220-4, CZL00009, CZL00001, CML205 and CML443 were good general combiners for GLS disease resistance, across the environments. In general, the good performance of the hybrids based on the SCA effects corresponded to at least one of the parental lines having a good GCA effect for disease resistance. Most of the hybrids which had CZL00009 and CZL00001 as resistant parents and crossed to susceptible parents such as N3 and A16 resulted in significant, negative SCA effects. The cross between N3 x CML205 resulted in the greatest amount of heterosis (-165 %). The same cross had relatively high negative estimates of SCA. Cross A16 x CML488 had significant negative SCA effects and relatively high amount of heterosis although its GLS score was on the moderately susceptible category. CML205 was amongst the good general combiners for disease resistance, whereas, N3 had the highest positive and significant estimates for GCA. These results showed that susceptible parents could be used in combination with resistant parents to produce resistant hybrids. Therefore, the significant SCA effects that were observed towards reduced disease imply that non-additive gene effects can be utilized in hybrid development.

6.5.1.3 Heterosis estimates

Negative mid-parent heterosis further confirmed the significance of non-additive effects in hybrid production. Negative mid-parent heterosis ranging from about 5% to 166% was observed in some of the hybrids. This amount of heterosis implied that non-additive gene action was important in the resistance for GLS. Results of this study are in agreement with observations made by Menkir and Ayodele (2005) and Derera *et al*. (2008) of the presence of negative heterosis exceeding 10% in some of the crosses which involved susceptible and resistant parents. Cromley *et al*. (2002) also reported similar results when crosses were made between resistant and susceptible temperate parents. The results confirm that adequate GLS resistance would be obtained in single cross hybrids when one parent is resistant.

6.5.2 Generation mean analysis

6.5.2.1 Disease development and frequency distributions

Disease rating done at the hard dough stage was used as this rating closely reflected the total damage to the leaf tissue for the entire growing season. Disease pressure was high as indicated by the high GLS scores recorded for the most susceptible parent, N3. The two susceptible parents differed in the degree of susceptibility, with N3 being more susceptible than B17 to GLS, thus indicating possibly different genetic backgrounds. There was good significant differentiation between the susceptible and resistant parents

involved in each of the crosses. This type of response indicated that differences observed in disease severity among the generations were due to genetic differences among parents. The resistant parents; A1220-4, A15 and CML441 used in this study can therefore be additional sources of resistance to GLS.

Variation in the segregating populations was approximately continuous and normal to slightly skewed for some of the crosses. This pattern was consistent with quantitative inheritance. This may indicate that several loci controlled the inheritance of GLS resistance.

6.5.2.2 Heterosis and average degree of dominance (ADD)

The F_1 and F'_1 generations for the A1220-4 x B17 and A15 x B17 had lower mean disease ratings than the mean of the two parents, indicating existence of some heterosis towards resistance. This was further confirmed by the mid-parent heterosis values which were also negative indicating resistance. The amount of heterosis only averaged -6% for these two crosses. Heterosis can be expressed when parents of a hybrid have different alleles at a locus and there is some dominance, over-dominance or epistasis among these alleles (Dabholkar, 1992; Falconer and Mackay, 1996). This confirmed that non-additive gene action had a role in the resistance to GLS in maize for these crosses. It also appears that for GLS resistance in maize, populations differ in terms of the non-additive effects. This was also confirmed by the results of the diallel analysis, where the range of mid-parent heterosis towards resistance was from -5% to -165%.

The average degree of dominance values in these three crosses ranged from -0.0 to 0.1, which is consistent with high levels of GCA and additive effects and the continuous distribution of GLS scores in the segregating populations. According to Edwards and Lamkey (2002), a value of 1 or -1 is considered to indicate complete dominance, whereas values of zero is no dominance and a value less than +1 or -1 shows incomplete dominance. Therefore, in this study the genes controlling resistance exhibited no dominance to incomplete dominance. These results were also corroborated by the non-significant dominance effects for two of the crosses; CML441 x N3 and A1220-4 x B17, and the low contribution of the dominance effects to the total variation among generations for A15 x B17. This observation contradicts findings by Hohls *et al*. (1995) who reported that resistance to GLS was conditioned by genes exhibiting complete dominance in a diallel comprising maize lines from three divergent backgrounds.

6.5.2.3 Genetic effects

The R^2 values for the genetic model were high (Table 6.9) indicating a very good fit for the model in all the three crosses. The three crosses had predominantly additive gene action. Close to ±90 % of the total variation for the generations was due to additive gene effects, confirming that resistance to GLS was predominantly additive. This is in agreement with what most investigators have reported (Thompson *et al*., 1987; Huff *et al*., 1988; Ulrich *et al*., 1990; Gevers and Lake 1994; Derera *et al*., 2008; Vivek *et al*., 2009) as well as the observation from the diallel analysis results in this current study. These significant additive genetic effects imply that selection for increased GLS resistance should be effective and the performance of the offspring predictable on the basis of the reaction of parents.

However, for A15 x B17 cross, dominance gene effects were also significant, whilst additive x additive and additive x dominance interactions were significant for CML441 x N3 and A1220-4 x B17 crosses, respectively. This result indicated that for some of the populations, in addition to additive genetic effects, dominance and epistasis were also important in the mechanism for resistance to GLS. The results also confirmed findings by other researchers using different populations who reported both additive and dominance gene action (Elwinger *et al*., 1990; Coates and White, 1998) and epistasis (Hohls *et al*., 1995) for GLS resistance. Nevertheless, the contributions of the dominance and epistatic effects to the total generation variation in the populations used in this current study were relatively small (5 to 7 %).

6.5.2.4 Heritability estimates and minimum number of genes (effective factors) controlling GLS resistance

The heritability estimates for the crosses indicated that GLS resistance was highly heritable and also indicated the degree of the differences between the parental lines in these crosses. The broad-sense heritability estimates from 64 to 92% observed in this study were within the range of heritability estimates reported for GLS in other studies (Clements *et al*., 2000; Vivek *et al*., 2001; Cromley *et al*., 2002; Derera, 2005). It has been observed that characters controlled by genes with additive effects have higher heritability estimates than those conditioned by genes with non-additive effects (Falconer and Mackay, 1996; Dabholkar, 1992). This implies that progress in selection would be made when heritability estimates are high, because there would be a close

correspondence between the genotype and the phenotype and the contribution of the environment to the phenotype would be small (Dabholkar, 1992).

All the crosses had minimum number of genes between two and three. Coates and White (1998), reported effective factors (minimum number of genes) of less than 5 for three of the five analysis they did in the populations they tested from American germplasm. The estimates of the number of genes observed in our study could have been biased downwards by the failure to meet the assumption of no dominance and no epistasis. Presence of epistasis and dominance has been reported to affect heritability estimates and number of effective factors (genes) (Fernandez and Miller, 1985). According to Hayman (1960), presence of epistasis is of major importance in the inheritance of a trait and results in biased estimates of pooled additive and dominance effects.

The assumption made in this GMA analysis was that the environmental variation was the same within each generation. However, failure to fulfil this assumption could have resulted in a biased effect on the results, including the heritability estimates. Increasing the number of replications and environments would result in the reduction of the environmental error and genetic x environment components in the phenotypic variance, which in turn improves the heritability estimate (Moreno-Gonzalez and Cubero, 1993). Although this experiment was not specifically designed to look at the genotype x environmental interactions, future studies will consider the effects of environmental variation on the inheritance of GLS disease through multi-location generation mean experiments.

6.6 Conclusion

The most resistant inbred lines A1220-4, CZL00009, CZL00001, CML205 and CML443 displayed good GCA for GLS resistance and contributed negative SCA effects in their respective crosses. Eight hybrids were resistant with GLS scores equal to or less than 4.0 and they had negative and significant SCA effects in addition to displaying high amounts of negative mid-parent heterosis in the F_1 hybrids. Two additional sources of resistance were CML441 and A15, which remained resistant even under high disease pressure in the GMA trial. Both additive and non-additive genes conditioned GLS resistance in the inbred lines. Results from both the diallel and the generation mean analysis supported the predominance of additive effects which accounted for 71% and 89%, respectively, of the variation for GLS resistance. Although small (equal to or more than 7%) non-additive

gene effects were significant in some crosses which exhibited dominance and epistasis for GLS resistance. In general, resistance was controlled by genes exhibiting zero dominance to partial dominance and was controlled by about two to three genes. There was no evidence to support a significant role of cytoplasmic gene effects because reciprocal F_1 crosses displayed similar levels of GLS resistance. Predominance of additive effects which is also reflected by high heritability (61 to 71%) suggests that GLS resistance could be enhanced by selection in some of the populations. Significant epistasis and dominance could however slow progress by compromising heritability estimates in some of the populations. This study also revealed that the use of one parent with resistance would provide adequate GLS resistance in single cross hybrids. Therefore, dominance effects towards reduced disease levels may be exploited in developing single cross hybrids for deployment in GLS prone environments.

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7 Diallel Analysis of Resistance to Northern Corn Leaf Blight and Common Rust Diseases in Tropical Advanced Maize Inbred Lines

Abstract

The incidence and severity of northern corn leaf blight (NLB) and common rust have increased in Southern Africa in the past three years and previously resistant cultivars are being affected. This means more sources of resistance have to be identified and their mode of gene action investigated. This study was therefore conducted to determine the combining ability, gene action and heterosis estimates for resistance to NLB and common rust among selected tropical advanced maize inbred lines. Forty five F_1 hybrids were generated by crossing ten inbred lines in a half diallel mating scheme. The 45 hybrids along with the ten inbred parents were evaluated in six environments for NLB and two environments for common rust, with two replications each between 2007 and 2009. General combining ability (GCA) and specific combining ability (SCA) effects were highly significant ($P \leq 0.001$) for NLB and common rust diseases and grain yield. GCA effects accounted for about 74% and SCA effects 26% of the variation in the hybrids for both NLB and common rust resistance, whereas both GCA and SCA effects for grain yield accounted for about 50% of the variation. This indicated the predominance of additive over non-additive gene action for the diseases and the importance of both additive and non-additive gene action for grain yield in these inbred lines. Overall, lines A16 and CML443 had good general combining ability for NLB and common rust resistance as well as high grain yield. The lines with negative GCA effects contributed towards NLB or common rust resistance in their hybrids even when crossed to lines with positive GCA effects. The significant additive effects for the diseases and grain yield entail that progress would be made through selection in breeding for disease resistance to NLB and common rust, and high grain yield. Although the non-additive gene effects might impede progress, they could be utilized in the development of hybrids with disease resistance and high grain yield. The results also showed that for both NLB and common rust diseases, it is possible to use a susceptible parent crossed with a resistant parent to produce resistant hybrids.

7.1 Introduction

The total annual maize production in sub-Saharan Africa (SSA) is estimated at approximately 34.42 million tonnes (Aquino *et al*., 2001). However, current average maize yields in the region remain low presenting a big challenge for researchers. Among the factors limiting maize productivity in SSA are pests and diseases (FAO and CIMMYT, 1997). Most of the diseases are difficult to control since their occurrence every season is less predictable because they depend very much on the prevailing weather conditions and are also influenced by the variable production environments in Africa. As a result, in favourable seasons with high rainfall, some diseases also become more prevalent and damaging. The majority of small-scale farmers cannot afford in most cases to control the diseases due to limited access to pesticides.

Northern corn leaf blight or turcicum blight (*Exserohilum turcicum* Pass. Leornard & Snuggs) and rust *(Puccinia sorghi* Schwein. and *P. polysora* Underw.) are amongst the diseases which are endemic to most SSA maize (Vivek *et al*., 2001). Lately, for example, there has been a resurgence of NLB in major maize growing areas in SSA. Vivek *et al*. (2009) also reported that incidence and severity of NLB had increased especially in Southern Africa in the past three years. This increase has the potential of threatening maize grain productivity with a negative impact on food security. Northern corn leaf blight has been reported to be severe in mid-altitude tropical regions as a result of high humidity, low temperature, and cloudy weather periods that prevail during the maize growing season. Yield losses of more than 50% can occur especially when the disease appears early before flowering (Raymundo and Hooker, 1981). This calls for additional sources of resistance to be made available to breeding research programmes.

Common rust, on the other hand, has the potential of causing yield losses which can exceed 34% in both the subtropics and highland tropical ecologies (Vivek *et al*., 2009). The disease has been reported to be particularly prevalent in higher elevations where temperatures are cooler. The common rust disease has also been observed in some of the germplasm adapted to African conditions concurrently on the same plants with other foliar diseases. Focus should therefore be in finding sources that are resistant to multiple diseases.

Additive gene action was found to be of major importance in most of the studies on the quantitative inheritance of NLB (Sigulas *et al*., 1988; Carson, 1995; Schechert *et al*., 1997; Vivek *et al*., 2009). However, significant dominance effects depended on the

genetic material tested and developmental stage of the plant, whereas epistatic gene action was not important (Schechert *et al*., 1997). Highly significant GCA effects were reported for common rust indicating the importance of additive gene action (Kim and Brewbaker, 1977; Paterniani *et al*., 2000; Vivek *et al*., 2009), although dominance was also significant in all the studies, its contribution was small. It would therefore be important to investigate the combining ability and gene action of any new sources of resistance that may be identified for the African environments, so as to come up with an appropriate breeding strategy.

Although, studies done on maize adapted to African conditions have identified some sources resistant to one or more of the endemic diseases, there are still few potential sources of resistance to multiple diseases in the region. Therefore, the development of maize cultivars with enhanced levels of disease resistance to individual and multiple diseases and greater abiotic stress tolerance will be more sustainable and effective for increased maize yields, especially in the smallholder-farming sector. This study was therefore conducted to: i) estimate the combining ability effects for resistance to NLB and common rust diseases among selected maize inbred lines ii) determine the gene action controlling NLB and common rust resistance, and iii) estimate heterosis on NLB and common rust resistance and grain yield in tropical African maize lines and their crosses

7.2 Materials and methods

7.2.1 Maize germplasm

Maize inbred lines were obtained from the CIMMYT programme in Harare, Zimbabwe, while the N3 inbred was obtained from the Crop Breeding Institute in Zimbabwe. The inbred lines were sampled from the major heterotic groups that are adapted to subtropical environments and are indicated in Table 7.1. Standard hybrid checks included were selected on the basis of their grain yield performance, stability and also resistance to a number of foliar diseases such as phaeosphaeria leaf spot (PLS), GLS, NLB and common rust. Hybrids that are also commonly grown by resource-poor smallholder farmers were also included.

Table 7.1 Designation, pedigrees and heterotic groups for parent inbred lines used in the diallel analysis

Designation†	Pedigree or Population (OPVs)	Heterotic grouping
A1220-4	[(CML395/CML444)-B-4-1-3-1-B/CML395//SC/ZM605#b-19-2-X]-1-2-X-1-1-BBBBBB]-7-1-3-2-BBB	B / SC
CML205	[EMSR]#B#bF101sr-2-1-sr-3-2-4-b-b	B
A16	Original pedigree CML312 (S89500F2-2-2-1-1-B*5)	A
CML445	[[TUXPSEQ]C1F2/P49-SR]F2-45-7-5-1-BBB	AB
CML488	DTPWC8F31-4-2-1-5-BBB	AB
CZL00001	INTA-191-2-1-2-BBBB	A
CZL00009	INTA-F2-192-2-1-1-1-BBBBB	A
MP18	[[[NAW5867/P30SR]-111-2/[NAW5867/P30SR]-25-1]-9-2-3-B-2-B/CML388]-B- 35-2-B-1-#-1-BB	A / P
N3	Salisbury White	N3
CML443	[AC8342/IKENNE{1}8149SR//PL9A]C1F1-500-4-X-1-1-BB-1-BB	AB

†some of the lines like A1220-4, A16 and MP18 were coded for convenience of study.

7.2.2 Diallel crosses and field evaluations

The ten advanced maize inbred lines listed in Table 7.1 were crossed in all possible combinations in a half-diallel mating scheme (excluding selfs) in 2006/7 season at the University of KwaZulu-Natal, Pietermaritzburg in South Africa. The resulting 45 single cross F_1 hybrids plus nine standard checks were evaluated in a total of six environments (year-location combinations) during 2007/8 and 2008/9 seasons. The parents were also evaluated in trials adjacent to the hybrid trials, but only in two environments. The locations included: Cedara (C), South Africa (30°16'E, 29°32'S, 1130 metres above sea level (m.a.s.l); Rattray Arnold Research Station (RARS), Zimbabwe (31°14'E, 17°40'S, 1300 m.a.s.l), and Mpongwe, Zambia (ZAMB) (28°8'E, 13°31'S, 1219 m.a.s.l). At Cedara, plantings were done in November 2007 (C108), January 2008 (C208) and November 2008 (C09). Plantings at RARS were in December, 2008 (RARS09) and Mpongwe, January 2008 (ZAMB08) and December 2008 (ZAMB09). The F_1 hybrids and standard hybrid checks were laid out in the field in two replications using a 9 x 6 alpha (0, 1) lattice design in each environment. Inbred parents were planted in a 10 x 2 randomised complete block design with two replications on the same day as the hybrids. The plot size for both the hybrids and parental lines in each environment was two rows, 3 m long, with 0.75 m inter-row spacing and 0.3 m intra-row spacing, except for Mpongwe where plots were one row, 5 m long with 0.75 m between rows and 0.3 m between the plants. Plant population densities were about 44 000 per hectare at all the environments. Fertiliser was applied at the rate of 120 kg N, 33 kg P, and 44 kg K. Standard cultural practices including ploughing and disking, hand planting, hand weeding and/or

application of herbicides and fertilizers were followed at each site and no fungicides application was done.

7.2.3 Disease assessment

Disease development was monitored on a fortnightly basis after the initial appearance of symptoms. NLB and common rust disease severity were then assessed twice, before flowering and at the hard dough stages, based on visual assessment of the whole plot using a 1-9 logarithmic rating scale, where 1 = 0% , 2 = <1%, 3 = 1-3%, 4 = 4-6%, 5 = 7-12%, 6 = 13-25%, 7 = 26-50%, 8 = 51-75% and 9 = 75-100% leaf area showing disease symptoms. The scores were further classified into the following disease reaction types; 1.0 = symptomless, 2.0-4.0 = resistant, 4.1-5.0 = moderately resistant, 5.1-6.0 = moderately susceptible, 6.1-9.0 = susceptible. The score recorded at the hard-dough stage was used for statistical analysis.

7.2.4 Other records

At harvest grain yield was measured on a whole plot basis (CIMMYT, 1985) and adjusted to 12.5% moisture (Zimbabwe Marketing Standards) using the formula:

Grain Yield (t ha^{-1}) = [Grain Weight (kg/plot) x 10 x (100-MC)/ (100-12.5)/(Plot Area)], where MC = Grain Moisture Content.

Heterosis for NLB scores, common rust scores and grain yield for each hybrid were estimated for the two environments that included parents, using mid-parent (MP) and better-parent (BP) scores (Falconer and Mackay, 1996) according to the following equations:

MPH (%) = 100*(F_1-MP)/MP, and BPH (%) = 100*(F_1-BP)/BP

Where F_1 = mean of the F_1 hybrid performance, MP = mean of the two parents making the cross and BP = mean of the better parent (resistant or high yielding) in the cross.

7.2.5 Data analysis

Mean disease ratings taken at the hard-dough stage of maize, grain yield and means of the other traits for the hybrids and standard checks were analysed using PROC GLM procedure in SAS 9.1 (SAS Institute, 2002). The data were subjected to ANOVA firstly by environment as the main effect, then a combined analysis across environments was conducted to analyse the effect of environments, hybrids and interactions. Standard checks were not included in the ANOVA for general combining ability (GCA) and specific combining ability (SCA) effects. Data were analysed for combining ability using the

Diallel SAS05 program in SAS (Zhang *et al.*, 2005).The F_1 hybrids were treated as fixed effects in the statistical analysis and environments (both spatial and temporal environments) as random effects. To estimate the GCA and SCA effects, Griffing's diallel analyses, Model 1 (fixed genotype effects); Method 4 (crosses only) was used according to the model:

$$Yijk = \mu + gi + gj + sij + eijk$$

Where*:*
Yijk = observed measurement for the ijth cross in the kth replication/ environment combination,
μ = overall mean,
gi and gj = GCA effects for the ith and jth parents, respectively,
sij = SCA effect for the ijth cross, and
eijk = error term associated with the ijth cross evaluated in the kth replication/environment combination.

The interaction terms were used to test for the significance of the corresponding main effects (Zhang and Kang, 1997). The environments and replications within environments were considered random and therefore tested against the residual error term.

7.3 Results

The results of the contrast of NLB severity scores and grain yield of the experimental hybrids versus checks are presented in Table 7.2. The entries (experimental hybrids plus checks), experimental hybrids, checks and their interactions with the environments were highly significant ($P<0.001$). The contrast of the NLB severity scores for the experimental hybrids against the checks was highly significant ($P<0.001$), but the interaction with the environment was not significant ($P>0.05$).

The entries (experimental hybrids plus checks) and their interaction with the environment were highly significant ($P<0.001$). The mean squares for experimental hybrids, checks and their interactions with the environments were also highly significant ($P<0.001$). The contrast of the grain yield for the experimental hybrids and checks was not significant ($P>0.05$), but its interaction with the environment was significant ($P<0.001$).

Table 7.2. Analysis of variance for NLB severity scores and grain yield over six environments: Experimental hybrids versus check hybrids.

		NLB Score	GrYld (t ha^{-1})
Source	DF	Mean Square	Mean Square
Environment	**5**	**97.49*** **	**456.85*** **
Rep(Env)	**6**	**0.90^{NS}**	**9.50*** **
Entry	**53**	**11.46*** **	**10.43*** **
Experimental hybrids (Exp)	44	11.19***	9.90***
Checks (Chks)	8	12.24***	12.31***
Exp vs Chks	1	17.19***	0.18^{NS}
Env*Entry	**265**	**2.00*** **	**3.81*** **
Env*Exp	220	1.93***	3.78***
Env*Chks	40	2.21***	3.48***
Env*Exp vs Chks	5	3.35^{NS}	10.73***
Error	**318**	**0.93**	**1.35**
Corrected Total	**647**		
Means			
Experimental hybrids	3.7		7.32
Hybrid Checks	4.2		7.28

*, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001, respectively, NS = non-significant (P>0.05).

7.3.1 Reaction of the inbreds and hybrids to NLB and common rust disease infection

Means for NLB disease, common rust disease and grain yield are presented in Tables 7.3 and 7.4. There were significant (P≤0.05) variation among the hybrids and inbreds for both diseases across the environments.

The NLB and common rust disease pressure was variable in the different environments as depicted by the different maximum scores recorded for the F_1 hybrids (Table 7.4). The ten inbred lines used as parents in this study showed significant differences (P≤0.05) in their reaction to the two foliar diseases. General reaction of the hybrids to the diseases in specific environments is shown in Fig 7.1a and 7.1b.

Mean scores for NLB amongst the inbred parents across the six environments ranged from 2.5 to 5.0 (Table 7.2). The most susceptible parents to NLB were MP18, CML205, A16, CML488 and A1220-4, with scores ranging from 6.0 to 8.5 (data not shown). The other inbred parents: CML443, CML445, CZL00001, CZL00009 and N3 were moderately

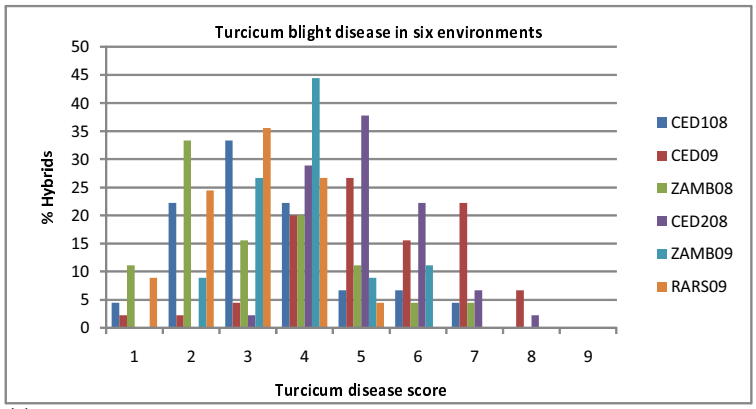
susceptible to resistant (scores 4.0 to 5.5). The most susceptible parents to common rust were CZL00001 with a mean score of 5.5 in C108 and N3 with a score of 4.5 in C09. The other inbred parents were all resistant with scores ranging from 1.0 to 2.5 in C108 and 1.0 to 3.0 in C09.

The F_1 hybrids were significantly different (P≤0.05) for NLB disease (Table 7.4) and common rust (Fig 1b) in the different environments. The hybrid checks varied in their reactions to NLB and common rust from resistant to susceptible depending on the environment. More than 60% of the hybrids were susceptible to NLB in the C09 and C208 environments than the rest of the other environments (Fig 7.1a). The NLB scores ranged from 3.0 to 7.5 in the C208 and from 1.0 to 8.0 in C09, with most of the hybrids showing a susceptible reaction. In the other environments, the scores were from 1.0 to 7.0 in C108 and ZAMB08, 2.0 to 6.0 in ZAMB09 and 1.0 to 5.0 in RARS09, with the most of the hybrids showing a resistant reaction (Fig 7.1a). In C108 hybrids ranged from 1.0 to 7.0 (Fig 7.1b). The overall frequency distribution of the hybrids across environments showed 70% of the hybrids having a score of equal to or less than 4.0.

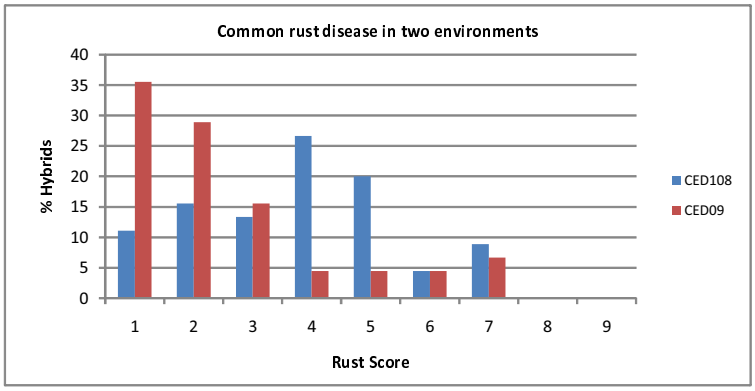
Differences were also observed for common rust disease severity in the two environments (Fig 7.1b).Disease scores in both C108 and C09 ranged from 1.0 to 7.0. However, in C108, the frequency distribution for the hybrids was approximately normal, whilst in C09 most of the hybrids had a resistant reaction. About 10% and 35% of the hybrids were symptomless at C108 and C09 environments, respectively. Fifty-percent of the hybrids in both C108 and C09 had a resistant score (equal to or less than 4.0).

Table 7.3 Means of parental lines for disease scores and grain yield of maize for the combined environments

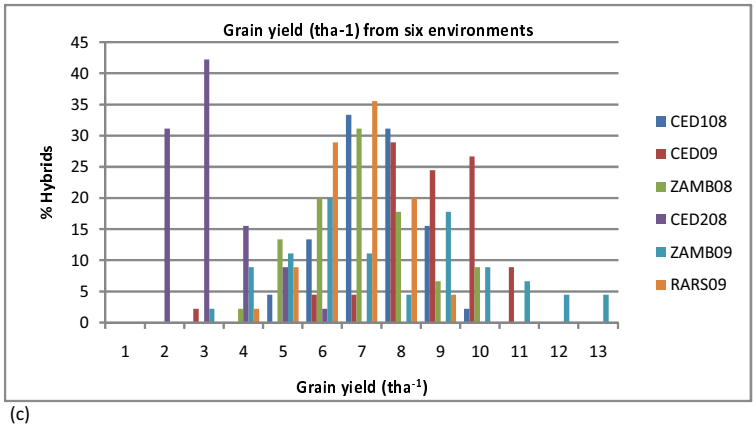
		Two environments	
Entry	Genoytpes	NLB	Common rust
1	CML445	4.3	1.0
11	A1220-4	3.8	1.8
20	CZL00009	3.8	1.3
28	CZL00001	3.5	4.3
35	N3	2.5	3.5
41	CML205	5.0	2.0
46	A16	4.8	1.8
50	MP18	4.8	1.0
53	CML443	4.0	1.3
55	CML488	3.8	1.8
Mean		4.0	2.0
LSD(0.05)		1.3	1.4



(a)



(b)



(c)

Figure 7.1 Frequency distribution of (a) NLB disease scores, (b) common rust disease scores and grain yield (t ha^{-1}) in the 45 F_1 maize hybrids evaluated in different environments between 2007 and 2009.

Table 7.4 Means of the F_1 hybrids and hybrid checks for NLB disease scores and grain yield of maize for the individual and combined environments

Entry	Cross	CED108	CED09	ZAMB08	CED208	ZAMB09	RARS09	Across Environments	Gr Yield
20 most resistant hybrids									
24	CZL00009xA16	1.0	4.5	1.0	3.0	1.5	1.0	2.0	9.4
37	N3xA16	1.5	1.0	1.0	5.0	2.5	2.0	2.2	7.3
26	CZL00009xCML443	2.0	2.5	2.5	4.0	2.0	1.0	2.3	8.4
12	A1220-4xCZL00009	2.5	4.0	1.0	4.5	1.5	1.0	2.4	7.8
19	A1220-4xCML488	1.5	3.5	2.0	4.0	3.0	1.0	2.5	7.9
13	A1220-4xCZL00001	1.5	3.0	2.0	4.0	3.0	2.5	2.7	8.5
34	CZL00001xCML488	2.5	5.0	1.0	3.5	2.5	2.5	2.8	7.1
18	A1220-4xCML443	2.5	5.5	2.0	3.5	2.0	2.0	2.9	7.3
16	A1220-4xA16	3.0	4.0	2.0	4.5	2.5	2.0	3.0	9.5
31	CZL00001xA16	2.5	4.5	1.5	4.5	2.5	2.5	3.0	8.8
33	CZL00001xCML443	3.0	3.5	2.5	5.0	2.5	1.5	3.0	7.6
27	CZL00009xCML488	2.0	5.5	1.5	3.5	3.0	3.5	3.2	6.2
2	CML445x A1220-4	1.5	6.0	2.0	5.0	3.5	1.5	3.3	7.8
7	CML445xA16	2.5	4.5	1.0	5.0	4.0	3.0	3.3	5.9
14	A1220-4xN3	2.5	2.0	4.0	5.0	3.5	3.0	3.3	8.3
21	CZL00009xCZL00001	2.0	5.5	2.0	4.0	3.0	3.5	3.3	8.5
23	CZL00009xCML205	3.0	4.5	1.5	4.0	4.0	3.0	3.3	6.2
29	CZL00001xN3	1.0	3.5	2.0	4.5	4.5	4.0	3.3	7.1
39	N3xCML443	3.0	4.0	2.0	4.5	4.0	2.5	3.3	7.1
54	CML443xCML488	2.5	5.0	1.5	6.0	3.5	1.5	3.3	6.3
Resistant checks									
63	P067	2.5	4.0	4.0	3.0	3.0	2.5	3.2	8.0
59	P77	3.5	4.0	4.0	5.0	2.5	2.0	3.3	6.2
62	S51	3.5	3.5	2.0	3.5	3.0	3.5	3.3	6.2
64	N72	4.5	4.0	3.5	3.5	3.0	2.0	3.4	8.0
60	S63	3.5	5.0	3.5	5.5	4.5	3.0	4.1	9.1
5 most susceptible hybrids									
38	N3xMP18	4.0	7.0	7.0	7.0	4.0	3.0	5.3	5.4
43	CML205xMP18	6.0	8.0	4.0	6.5	5.5	4.0	5.7	5.9
52	MP18xCML488	5.5	8.0	6.0	7.0	4.0	3.5	5.7	6.3
8	CML445xMP18	6.5	7.0	5.0	5.5	6.0	5.0	5.8	7.4
32	CZL00001xMP18	2.0	8.0	7.0	7.5	6.0	4.5	5.8	6.4
Susceptible checks									
61	S71	2.5	6.0	5.5	5.0	4.5	3.5	4.5	8.1
57	P27	3.0	5.5	4.0	7.0	4.5	4.0	4.7	7.2
56	P17	3.5	3.5	4.0	5.5	8.0	5.5	5.0	6.1
58	P57	5.5	8.0	5.5	7.0	5.0	5.5	*6.2*	6.4
Mean		3.2	5.1	3.2	4.8	3.7	2.8	3.8	7.1
LSD (0.05)		2.6	2.3	1.9	1.5	1.4	1.5	0.8	1.1

7.3.2 Grain Yield

There were significant differences (P≤0.05) in grain yield amongst the F_1 hybrids in the different environments (Fig 7.1c)). Yields were high in the normal season' plantings at

Cedara (C108 and C09), at Mpongwe (ZAMB08 and ZAMB09) and Rattray Arnold (RARS09) and ranged from 3.0 to 13.0 t ha^{-1} compared to about 2.0 to 6.0 t ha^{-1} for the late planting at Cedara (C208) (Fig 7.1c). The commercial hybrid S63 was amongst the highest yielding checks in all the environments with the exception of C208 where the yield was amongst the lowest. Almost 75% of the hybrids across the six environments had yields equal to or greater than 8.0 t ha^{-1}.

Table 7.5 Combined analysis of variance for grain yield (t $ha^{-1)}$, NLB and common rust disease severity scores of 45 F_1 hybrid crosses tested in different environments between 2007 and 2009 and the contribution of the different genetic effects to the total hybrid sum of squares.

	Six environments			Two environments		
		NLB score	Grain Yield (t ha^{-1})		Common rust score	Grain Yield (t ha^{-1})
Source	DF	MS	MS	DF	MS	MS
Environment (Env)	5	93.07***	356.14***	1	64.8	88.23***
Rep(Env)	6	1.35	8.39***	2	8.11	10.96***
Hybrid	44	11.19***	9.90***	44	9.94	3.68***
GCA	9	41.27***	24.14***	9	36.06	8.59***
SCA	35	3.46***	6.24***	35	3.23	2.41**
Env*Hybrid	220	1.93***	3.78***	44	1.97	2.69***
GCA*Env	45	3.66***	5.24***	9	3.66	4.00***
SCA*Env	175	1.49***	3.40***	35	1.53	2.35*
Error	264	0.93	1.37	88	1.00	
CV (%)		24.80	16.00		34.00	13.50
%GCA contribution (SS)		**75.40**	**49.90**		**74.10**	**47.70**
%SCA contribution (SS)		**24.60**	**50.10**		**25.90**	**52.20**

*, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001, respectively.

7.3.3 Combining Ability Effects and Gene Action

Mean Squares for environment, hybrid, general combining ability (GCA) effects, specific combining ability (SCA) effects and all the interactions were highly significant (P≤0.001) for both NLB and common rust diseases (Table 7.5). The GCA effects for NLB disease were three times larger than the SCA effects. The GCA effects contributed about 75% to the total hybrid sum of squares, while the SCA effects accounted for about 25% of the variation amongst the hybrids. The trend was similar for common rust disease, with the GCA effects being 2.8 times larger than the SCA effects. The common rust GCA effects accounted for 74% of the variation among hybrid common rust scores and the SCA accounted for about 26%.

Mean Squares for environment, hybrid, GCA and SCA effects for grain yield and the interactions were significant (P≤0.01). Across the two or six environments, the GCA effects for yield were slightly lower than the SCA effects. On partitioning the hybrid sum of squares for grain yield, the GCA effects accounted for about 48 to 50% and the SCA effects about 50 to 52% of the variation among the hybrids.

7.3.4 General Combining Ability Estimates of the Inbred Parents

The general combining ability (GCA) effects for disease scores and grain yield of the ten parents are presented in Table 7.6 and 7.7. The GCA effects for NLB and grain yield were highly significant (P≤0.001) in the different environments (Table 7.6).

For NLB, the GCA effects of the inbred parents were variable in the different environments (Table 7.6). Parents with significant (P≤0.01), negative GCA effects in more than one environment included: A1220-4, CZL00009, CZL00001, A16, CML443 and CML488. However, overall, CZL00001 and CML488 had a non-significant GCA effect. Parents with positive, significant GCA effects in more than one environment included: CML445, MP18 and CML205. The other parent, N3 had both negative and positive GCA effects depending on the environment.

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The GCA effects for grain yield also varied with the environments. Parents A1220-4, N3, CML205, A16, MP18, CML443 and CML488 all had significant (P≤0.05) GCA effects for grain yield. By and large, parents A1220-4 and A16 had significant, positive GCA effects, while parents CML205, MP18 and CML488 had negative GCA effects for yield.

The GCA effects for common rust disease were variable in the two environments (Table 7.7). Parents CML445, A16 and CML443 had negative, significant (P≤0.001) effects across the environments, whereas positive, significant (P≤0.01) GCA effects were observed for A1220-4, and CZL00001 and CZL00009 across the environments. Parents A1220-4 and CML443 had positive, significant (P≤0.05) GCA effects for grain yield across the two environments, whereas MP18 had negative GCA effects (Table 7.7).

Table 7.6. Estimates of general combining ability (GCA)† effects for NLB and grain yield (t ha^{-1}) evaluated in six environments between 2007 and 2009.

NLB		Environments						
Entry #	Parent	C108	C09	ZAMB08	C208	ZAMB09	RARS09	Combined
1	CML445	0.31	0.63*	-0.48*	0.09	0.83***	0.51**	0.31*
11	A1220-4	-0.63*	-1.06***	-0.29	-0.48**	-0.61***	-0.93***	-0.66***
20	CZL00009	-0.31	-0.25	-0.73**	-0.48**	-0.80***	-0.43*	-0.50***
28	CZL00001	-0.88**	-0.19	-0.48*	-0.16	0.14	0.45*	-0.19
35	N3	-0.13	-1.38***	0.03	0.65***	0.20	0.26	-0.06
41	CML205	0.56	0.63*	0.21	-0.29	0.83***	0.76***	0.45***
46	A16	-0.50	-0.56*	-0.35	-0.23	-0.61***	-0.55**	-0.47***
50	MP18	2.00***	1.81***	2.84***	1.21***	0.95***	0.64***	1.58***
53	CML443	-0.31	0.13	-0.23	-0.54**	-0.61***	-0.61**	-0.36**
55	CML488	-0.13	0.25	-0.54*	0.21	-0.30*	-0.11	-0.1

GCA for grain yield (t ha^{-1})		Environments						
Entry #	Parent	C108	C09	ZAMB08	C208	ZAMB09	RARS09	Combined
1	CML445	-0.26	0.22	0.00	-0.13	0.26	-0.01	0.01
11	A1220-4	0.25***	0.84***	0.75**	0.59*	1.31***	0.39	0.69***
20	CZL00009	0.12	0.32	0.01	-0.10	1.14***	0.34	0.31
28	CZL00001	-0.19*	0.39	-0.34	0.49	1.46***	0.21	0.34
35	N3	0.85***	-0.74**	1.30***	-0.17	-0.65*	-0.79***	-0.03
41	CML205	-0.45*	-0.44	-1.01***	-0.35	-1.85***	0.14	-0.66***
46	A16	0.48**	0.38	1.17***	0.80***	0.38	0.41	0.60**
50	MP18	-0.69	-1.50***	-0.90***	-0.77**	-0.61	-0.05	-0.75**
53	CML443	0.54	0.62*	-0.11	-0.50	-0.22	-0.60**	-0.04
55	CML488	-0.65	-0.08	-0.87**	0.14	-1.23***	-0.06	-0.46*

†Negative GCA effects were desirable for NLB resistance, whereas positive GCA effects were desirable for high grain yield. *, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001

Table 7.7. Estimates of general combining ability (GCA)† effects for common rust disease and grain yield (t ha^{-1}) evaluated in two environments at Cedara between 2007 and 2009.

		Environment			Grain Yield
Entry #	Parent	C108	C09	Across Environments	Across Environments
1	CML445	-1.41***	-1.31***	-1.41***	-0.02
2	A1220-4	0.78***	1.88***	0.78***	0.54*
3	CZL00009	0.59**	0.13	0.59**	0.22
4	CZL00001	2.15***	1.88***	2.15***	0.10
5	N3	0.21	0.06	0.21	0.05
6	CML205	0.40	0.06	0.40	-0.45
7	A16	-1.04***	-0.25	-1.04***	0.43
8	MP18	0.21	-1.06***	0.21	-1.10***
9	CML443	-1.48***	-1.00***	-1.48***	0.58*
10	CML488	-0.41	-0.38	-0.41	-0.37

†Negative GCA effects were desirable for common rust resistance, whereas positive GCA effects were desirable for high grain yield. *, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001

7.3.5 Specific combining ability estimates for the diseases and grain yield

Specific combining ability (SCA) estimates for the two diseases in the different environments are shown in Tables 7.8 and 7.9. The effects were variable in the different

environments. In general, only a few hybrids had significant SCA effects in the six environments. Hybrids with negative, significant (P≤0.05) SCA effects for NLB ranged between one and four in the different environments. On the whole, CZL00009 x A16, N3 x A16 and MP18 x CML443 had negative, significant (P≤0.05) SCA effects across the environments.

Two hybrids had significant (P≤0.05) negative SCA effects for common rust disease in the two environments and these were CML445 x A1220-4 and CZL00001 x CML488 (Table 7.9). Negative SCA effects were observed for hybrids which involved mainly CML445, CML205 and CML443 as parents. Hybrid CML443 x CML488 was the only one with significant, negative SCA effects for common rust disease across the environments.

Specific combining ability (SCA) effects for grain yield are presented in Table 7.10. The SCA effects were highly variable in the different environments. Hybrids with significant (P≤0.05) positive SCA effects for grain yield varied from one to eleven in the different environments. However, only two hybrids had significant SCA effects for grain yield across the environments and these were CML445 x A16 (negative SCA effects) and CML205 x N3 (positive SCA effects).

Table 7.8 Hybrids with significant estimates of specific combining ability (SCA) effects for NLB disease in six environments for the set of diallel crosses among ten maize inbred lines

Entry #	Cross	NLB Mean Score	Environment						
			C108	C09	ZAMB08	C208	ZAMB09	RARS09	Across Environments
3	CML445xCZL00009	4.4	-0.17	0.96	2.22***	1.08**	0.38	0.70	0.86*
8	CML445xMP18	5.8	1.02	-0.60	-0.34	-0.61	0.63	1.14*	0.20
14	A1220-4xN3	3.3	0.08	-0.73	1.28*	0.01	0.31	0.95	0.32
22	CZL00009xN3	3.8	0.77	2.96***	0.22	0.51	0.00	-0.55	0.65
24	CZL00009xA16	2.0	-1.35	0.15	-0.90	-1.11**	-0.69	-0.74	-0.77*
25	CZL00009xMP18	4.8	2.15***	-1.73**	-0.09	-0.05	0.25	-0.42	0.02
26	CZL00009xCML443	2.3	-0.54	-2.54***	0.47	0.20	-0.19	-0.67	-0.55
27	CZL00009xCML488	3.2	-0.73	0.33	-0.22	-1.05**	0.50	1.33**	0.03
30	CZL00001xCML205	4.6	0.15	1.40*	0.78	0.14	0.94*	0.08	0.58
32	CZL00001xMP18	5.8	-2.29***	1.21	1.66**	1.64***	1.31***	0.70	0.70
33	CZL00001xCML443	3.0	1.02	-1.60*	0.22	0.89*	-0.63	-1.05*	-0.19
34	CZL00001xCML488	2.8	0.33	-0.23	-0.97	-1.36***	-0.94*	-0.55	-0.62
37	N3xA16	2.2	-1.04	-2.23***	-1.65**	-0.24	-0.69	-0.42**	-1.05**
38	N3xMP18	5.3	-1.04	1.40*	1.16	0.33	-0.75	-0.61	0.08
39	N3xCML443	3.3	0.27	0.08	-0.78	-0.42	0.81*	0.14	0.02
40	N3xCML488	3.6	0.58	-0.04	0.03	-0.17	-1.00**	0.64	0.01
43	CML205xMP18	5.7	0.27	0.40	-2.03***	0.76	0.13	-0.11	-0.10
45	CML205xCML488	3.8	0.40	-1.04	-0.15	0.26	-0.63	-0.36	-0.25
48	A16xCML443	4.2	1.65*	1.27	2.60***	0.45	1.13***	0.45***	1.26***
49	A16xCML488	4.4	0.96	2.15***	1.91***	1.20***	1.31***	-0.05****	1.25***
51	MP18xCML443	4.2	-1.35	-0.10	-1.09	-1.99***	-0.94*	0.76	-0.78*
54	CML443xCML488	3.3	-0.23	-0.54	-0.72	1.51***	0.81*	-0.49	0.06

†Negative GCA effects were desirable. *, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001

Table 7.9 Hybrids with significant estimates of specific combining ability (SCA) effects for common rust disease in two environments for the set of diallel crosses among ten maize inbred lines

Entry #	Cross	Common rust Mean Score	Environment			Grain Yield across environments
			C108	C09	Across Environments	
2	CML445x A1220-4	1.5	-1.40*	-1.40*	0.01	-0.28
3	CML445xCZL00009	1.0	-1.71***	-0.15	0.00	0.57
4	CML445xCZL00001	2.5	-0.77	-1.40*	0.00	-0.12
7	CML445xA16	1.0	-0.08	0.23	0.00	-2.52***
8	CML445xMP18	2.5	1.67***	1.04	0.01	0.50
9	CML445xCML443	1.5	0.85	1.48*	-0.02	0.58
18	A1220-4xCML443	1.8	-0.33	-2.21***	-0.01	-0.86
19	A1220-4xCML488	4.8	0.10	1.67**	0.01	0.40
21	CZL00009xCZL00001	6.8	0.73	2.17***	-0.02	-0.31
24	CZL00009xA16	3.5	1.92***	-0.21	-0.01	0.35
25	CZL00009xMP18	2.0	-1.33**	-0.40	0.02	-0.17
26	CZL00009xCML443	1.8	-0.65	0.04	0.04**	0.12
27	CZL00009xCML488	3.5	1.79***	-0.58	0.01	-1.06
29	CZL00001xN3	6.5	0.60	2.23***	-0.01	-0.31
30	CZL00001xCML205	6.0	0.42	1.23*	0.01	0.14
32	CZL00001xMP18	3.5	0.10	-2.15***	0.00	0.14
34	CZL00001xCML488	3.0	-1.77***	-1.33*	0.01	0.10
37	N3xA16	2.0	-0.21	-0.65	0.03*	-0.65
38	N3xMP18	2.5	-0.46	0.17	-0.01	-1.54*
39	N3xCML443	1.0	-1.27*	-0.40	-0.01	0.70
42	CML205xA16	1.5	-1.40**	-0.65	-0.01	0.13
44	CML205xCML443	3.3	1.54**	1.10	0.04**	-0.40
45	CML205xCML488	1.5	-1.52**	-1.02	-0.02	-0.26
51	MP18xCML443	1.0	-1.27*	0.73	0.00	0.64
54	CML443xCML488	1.8	0.85	0.04	-0.03**	-0.94

†Negative GCA effects were desirable. *, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001

Table 7.10 Hybrids with significant estimates of specific combining ability (SCA) effects for grain yield in six environments for the set of diallel crosses among ten maize inbred lines

Entry	Cross	C108	C09	ZAMB08	C208	ZAMB09	RARS09	Across Environments
2	CML445x A1220-4	-1.05	0.49	-1.90***	0.15	0.28	0.48	-0.26
7	CML445xA16	-1.51**	-3.53***	0.06	0.05	-5.74***	-1.49**	-2.03***
8	CML445xMP18	0.15	0.85	0.44	-0.58	3.84***	0.17	0.81
9	CML445xCML443	1.83***	-0.66	0.09	0.75	0.71	0.32	0.50
10	CML445xCML488	0.25	2.09***	1.10	-0.08	0.87	-0.27	0.66
12	A1220-4xCZL00009	0.99	-0.21	2.06***	-1.37	-3.21***	-1.17*	-0.49
15	A1220-4xCML205	-0.49	1.18	-2.36***	1.58*	-1.57	-0.17	-0.30
16	A1220-4xA16	0.01	0.98	0.72	0.71	2.55**	0.21	0.86
17	A1220-4xMP18	-1.56**	-0.02	-0.16	0.08	3.04***	-0.63	0.13
18	A1220-4xCML443	-0.46	-1.27	-0.64	-0.15	-2.00**	0.27	-0.71
21	CZL00009xCZL00001	0.69	-1.32	0.76	0.30	2.94***	-0.24	0.52
22	CZL00009xN3	-0.15	1.18	-0.55	-0.09	1.75*	-0.34	0.30
23	CZL00009xCML205	0.07	-0.86	-0.02	-0.50	-2.75***	-0.47	-0.75
24	CZL00009xA16	0.77	-0.08	0.04	1.79**	2.57**	1.91***	1.17
25	CZL00009xMP18	-0.97	0.63	-1.04	-0.32	-3.09***	-0.18	-0.83
26	CZL00009xCML443	0.01	0.24	-0.06	0.45	4.02***	0.02	0.78
27	CZL00009xCML488	-2.08***	-0.04	-0.17	-0.61	-2.82***	-0.27	-1.00
29	CZL00001xN3	-1.01	0.40	-1.90***	-0.02	0.38	-0.81	-0.49
30	CZL00001xCML205	-0.05	0.34	1.72**	-0.36	-1.37	-0.99	-0.12
31	CZL00001xA16	0.47	0.73	1.03	-1.54*	1.65*	0.84	0.53
32	CZL00001xMP18	-0.27	0.55	-0.52	-0.01	-2.91***	0.26	-0.48
33	CZL00001xCML443	-1.35*	0.12	-0.95	0.27	0.25	1.30*	-0.06
34	CZL00001xCML488	0.78	-0.58	-0.53	1.47*	-1.09	-0.64	-0.10
36	N3xCML205	0.68	1.10	-0.69	-0.80	4.29***	2.61***	1.20*
38	N3xMP18	0.09	-3.18***	-0.28	-0.51	-2.50**	-0.39	-1.13
39	N3xCML443	-0.45	1.84**	0.97	-0.10	-1.79*	-1.35*	-0.15
40	N3xCML488	0.09	-0.29	0.37	2.04***	0.53	-0.44	0.38
42	CML205xA16	0.66	-0.39	-0.04	-0.81	3.66***	-0.54	0.42
44	CML205xCML443	-0.72	-0.08	0.05	0.89	-1.69*	-0.73	-0.38
51	MP18xCML443	0.89	0.38	2.25	-0.09	2.67***	0.01	1.02
54	CML443xCML488	0.10	-1.97**	-0.79	-0.63	-0.66	0.77	-0.53

Positive effects were desirable for high grain yield. *, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001

7.3.6 Estimates of heterosis

Mid-parent heterosis (MPH) and better-parent heterosis (BPH) estimates for grain yield are presented in Table 7.11. Both the MPH and BPH estimates were variable among the hybrids. The MPH and BPH were all positive and ranged from 64 to 422% and 13 to 372%, respectively. The highest amount of heterosis was observed in A1220-4 x A16 hybrid, whilst the lowest amount of heterosis was in the N3 x MP18 hybrids. Generally, hybrids involving parental lines A1220-4 and A16 had higher heterosis estimates than the other parental lines.

7.3.7 Phenotypic correlations

The correlations among NLB disease scores among environments were significant and positive for all the environments, except RARS09 and C208. Common rust scores were positively correlated in the two (C108 and C09) environments ($r = 0.67$, $P \leq 0.001$, data not shown). Significant correlations ($P \leq 0.01$) for GCA effects were detected between NLB and grain yield ($r = -0.80$, Table 7.12). All the correlations between common rust and yield were not significant ($P > 0.05$, Table 7.13).

Table 7.11 Estimates of percentage mid-parent and better-parent heterosis for grain yield (tha^{-1}), NLB and common rust diseases for the 45 F_1 hybrids evaluated between 2007 and 2009.

Parent	Grain Yield (tha^{-1})	
	%MPH	%BPH
CML445x A1220-4	189.5	122.2
CML445xCZL00009	136.5	135.2
CML445xCZL00001	136.5	115.2
CML445xN3	96.7	71.3
CML445xCML205	166.1	93.0
CML445xA16	128.5	63.7
CML445xMP18	166.9	100.8
CML445xCML443	148.4	144.7
CML445xCML488	200.1	135.7
A1220-4xCZL00009	221.2	147.5
A1220-4xCZL00001	256.3	194.5
A1220-4xN3	143.6	70.2
A1220-4xCML205	360.7	324.4
A1220-4xA16	422.0	372.0
A1220-4xMP18	251.4	241.4
A1220-4xCML443	196.0	129.6
A1220-4xCML488	316.8	303.9
CZL00009xCZL00001	139.1	118.6
CZL00009xN3	101.8	74.9
CZL00009xCML205	178.9	103.0
CZL00009xA16	239.0	143.6
CZL00009xMP18	154.5	92.2
CZL00009xCML443	144.2	141.8
CZL00009xCML488	138.8	88.3
CZL00001xN3	95.9	57.6
CZL00001xCML205	232.4	157.6
CZL00001xA16	290.0	198.0
CZL00001xMP18	194.1	137.7
CZL00001xCML443	143.4	124.5
CZL00001xCML488	205.0	158.7
N3xCML205	154.2	69.6
N3xA16	138.4	57.5
N3xMP18	64.1	12.8
N3xCML443	115.1	85.0
N3xCML488	114.8	52.9
CML205xA16	396.0	386.1
CML205xMP18	272.7	252.8
CML205xCML443	195.0	116.1
CML205xCML488	270.9	232.0
A16xMP18	361.7	328.8
A16xCML443	271.5	168.6
A16xCML488	341.0	287.7
MP18xCML443	197.3	126.1
MP18xCML488	259.8	239.1
CML443xCML488	157.4	104.4
Mean	202.0	152.9
LSD (0.05)	12.3	9.8

Table 7.12 Pearson correlation coefficients for NLB scores (above diagonal) and Grain Yield (below diagonal) among environments which had significant differences

	C108	C09	ZAMB08	C208	ZAMB09	RARS09
C108	1	0.42**	0.59***	0.46***	0.48***	0.30*
C09	0.16	1	0.53***	0.34*	0.45***	0.43**
ZAMB08	0.62***	0.13	1	0.59***	0.52***	0.35*
C208	0.12	0.24	0.12	1	0.52***	0.25
ZAMB09	0.32*	0.43**	0.24	0.20	1	0.70***
RARS09	0.03	0.26	-0.15	0.20	0.44**	1

*, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001

Table 7.13 Pearson correlation coefficients among SCA effects (above diagonal) for 45 F_1 hybrids and GCA effects (below diagonal) for 10 inbred parent lines for the NLB, common rust diseases and grain yield evaluated in different environments

	Yield	NLB	Common rust
Yield		-0.11	-0.01
NLB	-0.80***		-0.17
Common rust	0.16	-0.06	

*, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001

7.4 Discussion

7.4.1 Disease development

The significant hybrid main effects and interactions for the two diseases indicated that the hybrids were different and the environments diverse. The significant GCA and SCA effects observed showed that both additive and non-additive gene effects were important in the resistance to NLB and common rust diseases. For both diseases the GCA effects were higher than the SCA effects indicating that additive gene action was more predominant than non-additive gene action. This implies, therefore, parents of the crosses can be selected *per se* based on their reaction to the diseases. This would ensure efficient use of resources than estimating the GCA effects first. This result is in agreement with observations made in almost all the studies on the quantitative inheritance of NLB using various inbred lines (Sigulas *et al*., 1988; Carson, 1995; Schechert *et al*., 1997; Vivek *et al*., 2009), which reported that additive gene action played a major role in the resistance. Significant dominance effects were also reported but they depended on the genetic material tested, whereas epistatic gene action was not important (Schechert *et al*., 1997). In this study, non-additive gene action was also important, but its contribution was small, compared to the additive gene effects. Breeders can, however, take advantage of this non-additive gene action that was associated with reduced disease levels by developing single cross maize hybrids among these inbreds.

Highly significant GCA effects observed for common rust in this study are also in agreement with the finding by other researchers who reported the importance of additive gene action for common rust (Kim and Brewbaker, 1977; Paterniani *et al*., 2000; Vivek *et al*., 2009) amongst various inbred lines. In this study, although dominance was also significant its contribution was small, and this observation was similar to that made by Kim and Brewbaker (1977), Paterniani *et al*. (2000) and Vivek *et al*. (2009).

The significant interactions indicated that both hybrids and inbreds were highly variable in their responses to the different environmental changes. However, correlation coefficients of NLB and common rust disease scores with environments, showed almost all the environments to be positively correlated with the exception of C208 with RARS09 for NLB.

This means that these environments provided a good genetic discrimination of the inbreds and hybrids to both diseases. Consequently, evaluation for these two diseases in any one of these environments would be sufficient to select resistant germplasm. Therefore, in order to reduce costs in disease screening, one can select for NLB or common rust resistance at one reliable site and still be able to distribute the lines or hybrids to other environments. This result is in agreement with the observation made by other researchers for various diseases, such as grey leaf spot (Lipps *et al*., 1998, Derera *et al*., 2008; Vivek *at al*., 2009) and for maize streak virus (Mawere *et al*., 2006) for different environments.

7.4.2 Reaction of inbred parents to NLB and common rust diseases and combining ability effects

The most resistant parents for NLB were A1220-4, CZL00009, CZL00001, N3, A16, CML443 and CML488. However, from this group of parents, only A1220-4, CZL00009 and CZL00001 had negative significant GCA effects. Most of the inbred parents were resistant to common rust disease. The most susceptible parent to common rust was CZL00001 with a mean score of 5.5 in C108, but 3.0 in C09, which implies that it is probably moderately resistant to susceptible, maybe depending on the disease pressure. The lines which had negative GCA effects for common rust were CML445, A16 and CML443.

7.4.3 Reaction of hybrids to NLB and common rust diseases and combining ability effects

Significant variation amongst the hybrids to the two diseases was observed. The results indicated that disease pressure for NLB was high at Cedara for C09 and C208. The three hybrids; CZL00009 (R) x A16, N3 (R) x A16 (S) and MP18 (S) x CML443 (R) which had significant SCA effects across the environments involved resistant (N3, and CML443) and susceptible parents (A16 and MP18). Parent A16 and CML443 had significant negative GCA effects, and contributed towards resistance in these crosses.

The lines with negative GCA effects contributed towards common rust resistance in their hybrids in the various environments, even when crossed to lines with positive GCA effects. However, when environments were combined, hybrid CML443 x CML488 was the only one

with significant, negative SCA effects for common rust disease for the combined environments. Both CML443 and CML488 were resistant to common rust and had negative GCA effects across the environments.

7.4.4 Combining ability effects for grain yield

The significant hybrid, GCA and SCA effects for grain yield indicated that both additive and non-additive gene action were important for grain yield. The significant interactions with the environment for hybrids, GCA and SCA effects imply that the inbreds or hybrids in one location may have a different reaction for yield in another location. The breeding strategy would therefore be to breed for specific adaptation. The GCA and SCA effects were both important for grain yield. This implied both additive gene and non-additive gene action were important for grain yield in these inbred lines. Therefore, breeding strategies that take advantage of heterosis and the general and specific combining abilities would more appropriate for increasing grain yield (Moreno-Gonzalez and Cubero, 1993).

There were significant differences in grain yield amongst the F_1 hybrids in the different environments. The late planting (C208) at Cedara had lower yields than the early planted crop (C108 and C09) and ZAMB08. The late planted crop was probably affected by the rainfall distribution during the growing season and also a short season. A mid-season drought was experienced in this environment from the mid-January 2008 till the end of March 2008 and no supplementary irrigation was applied. Most of the hybrids performed poorly in C208 environment compared to C108, C09 and ZAMB08 enviroments. Most of these hybrids seem to be adapted to high potential environments only. The short growing season could have disadvantaged the medium to late maturing hybrids resulting in low yields. Therefore, C208 environment was appropriate for selection of hybrids that perform well under drought-stress environments and short-season hybrids. The drought-stress affected the plants mostly before and around flowering. However, the frequency distribution for grain yield for the combined environments indicated the majority of the experimental hybrids were high yielding.

Parents A1220-4 and A16 were the only ones with positive significant GCA effects for grain yield for the combined environments, an indication that they would contribute towards higher

yields in the hybrids they were involved. Although these parents contributed towards high yield in some of the hybrids in the different environments, only one hybrid (N3 x CML205) had significant positive SCA effects when the environments were combined. This showed the importance of genes with non-additive effects for grain yield in these inbred lines. Significant correlations (P≤0.01) for GCA effects were also detected between NLB and grain yield (r = -0.84). This implies that NLB can negatively affect yield, confirming the potential that the disease has of causing significant yield reductions.

7.4.5 Estimates of heterosis

Mid-parent and better-parent heterosis was observed for grain yield. The amount of heterosis was specific to each cross. Significant positive heterosis for grain yield was desirable and generally, most of the hybrids had high heterosis estimates. This further confirmed that genes with non-additive effects were important in the grain yield of the hybrids. Most of the hybrids would actually be quite useful to the farmers and should therefore be recommended for further testing. According to Sleper and Poehlman (2006), for a hybrid plant to be useful to the farmer or breeder, it had to exceed the best parent in yield and productivity.

7.5 Conclusion

The resistant inbred lines to NLB were A1220-4, CZL00009, CZL00001, N3, CML443 and CML488. However, from this group of lines, only A1220-4, CZL00009 and CML443 had good general combining ability for NLB resistance. Line A16 was moderately resistant but it had desirable negative GCA for NLB resistance. Almost all the inbred lines used in this study were resistant or moderately resistant to common rust. However, only CML445, N3, A16 and CML443 had negative GCA for common rust resistance. Parents A1220-4, A16 and CML443 had in general positive GCA for high grain yield and these lines also contributed towards high positive heterosis in most of the hybrids they were involved. Overall, lines A16 and CML443 had good GCA for NLB and common rust resistance as well as high grain yield.

General combining ability accounted for more than 74% and the SCA effects about 26% of the hybrid sum of squares for NLB and common rust diseases, indicating the predominance

of additive over non-additive gene action. In contrast, for grain yield, both GCA and SCA effects were equally important. Largely, the highly significant additive effects for the diseases suggest that progress would be made through selection. Resistance can be incorporated through methods such as backcross or recurrent selection in these inbred lines. However, the important non-additive gene effects which might slow progress can be used in the development of hybrids with disease resistance.

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8 Genotype-Environment Interaction and Grain Yield Stability of African Maize Germplasm across different Stress Environments

Abstract

The highly variable environments in SSA contribute to complicated genotype x environment interactions (GEI). Multi-environmental trials (METs) would therefore be useful to identify superior varieties that can be recommended to the farmers. Therefore, the objectives of the study were to evaluate the level of grain yield stability and identify the best performing genotypes for wide and specific adaptation in different African environments. Forty five F_1 hybrids were generated by crossing ten inbred lines in a half diallel mating scheme. The 45 hybrids along with nine hybrid checks were evaluated across 11 environments varying in moisture and disease levels, with two replications each between 2007 and 2009. Additive Main Effects and Multiplicative Interaction (AMMI) and the genotype and genotype by environment (GGE) biplot analyses were used in identifying the superior genotypes. Common hybrids selected by AMMI and GGE biplot were H21 (CZL00009 x A16), H14 (A1220-4 x A16), S63 (SeedCo hybrid check), N72 (MP72/N3) and H26 (CZL00001 x A16). Hybrids H1 (CML445 x A1220-4), H44 (CZL00009 x CML443), and H18 (CZL00009 x CZL00001) were identified by both AMMI and GGE biplot analyses as unstable but high yielding. The GGE biplots gave more visual interpretations than just selecting the best performing hybrids and allowed visualization of crossover GEI through the polygon view. AMMI analysis identified ZAM08, C108, BF09, RA09 and C09 as the high yielding environments which were relatively stable (had IPCA1 scores between 0 and ±0.5). GGE biplots selected KD09, ZAM08, C208, C108, UG09, C09 and RA09 as the most representative environments (had PC1 and PC2 scores close to zero), indicating stability and high yield. Overall, the AMMI and GGE biplot analyses resulted in more or less similar selections of superior, stable hybrids and best performing environments.

8.1 Introduction

Selection of genotypes for wide adaptability is often limited by the existence of genotype by environment interaction (GEI). A significant GEI would mean that selections from one environment may perform poorly in another (Fox *et al.*, 1997). This would necessitate breeding for specific adaptation. In addition, the GEI means that it would be difficult to predict a response, thereby complicating the process of selecting genotypes with superior performance (Dudley and Moll 1969). This tends to slow progress from selection, since different genotypes would have to be chosen in different environments (Dudley and Moll, 1969). Consequently, multi environmental trials (METs) have been used to identify the varieties which are superior and can be recommended to farmers. METs also assist in the identification of production environments that best suit certain genotypes (Yan *et al*., 2001). It is, therefore, important to identify the causes of GEI in order to set up appropriate breeding objectives.

These GEI have different implications on the choice of genotypes. In general, GEI can cause changes in the ranking of the genotypes in different environments or may result in the genotypes behaving differently but without changes in the rank order in the different environments. A change in rank order is defined as cross-over interaction and is a major problem in breeding (Cooper and Delacy, 1994; Crossa *et al*., 1991), because it can slow down selection progress as different cultivars are selected in different environments. Genotypes that show little interaction with environments are often desired by plant breeders as they are stable (Tollenaar and Lee, 2002). Stability has been shown to be either static or dynamic (Becker and Leon, 1988). Static stability results in the performance of the genotype not changing even when the environmental conditions change. On the other hand, dynamic stability is when the performance of the genotype changes across a wide range of environmental conditions, but in a way that is predictable.

Different methods have been used to explore GEI and identify superior genotypes with wide or specific adaptation for different environments. Currently most breeders are using the additive main effects and multiplicative interaction (AMMI) analysis (Gauch, 1992; Gauch and Zobel, 1997; Zobel *et al*., 1988) and the genotype and genotype by environment (GGE) biplot

analysis (Yan and Kang, 2003; Yan and Tinker, 2005; Yan *et al*., 2007). The other traditional methods include: ANOVA (Snedecor and Cochran, 1980), linear regression (Finlay and Wilkinson, 1963; Eberhart and Russel, 1966) and principal component analysis (Hill and Goodchild, 1981). The advantages and disadvantages of the AMMI and GGE biplot analyses are dealt with in detail by Gauch (2006) and Yan *et al.* (2007). The main difference between the two analyses being that in AMMI biplots the genotype main effect is included as a multiplicative effect and not as an additive main effect (Yan and Kang, 2003). In this study both the AMMI and GGE biplot analyses were used.

The objectives of the study were to: i) evaluate the level of grain yield stability in ten advanced tropical inbred lines under mid-altitude tropical environments, ii) identify the best performing genotypes in terms of grain yield across or in specific environments, and iii) examine and compare the results obtained by AMMI and GGE biplot analyses in identifying grain yield superior genotypes in the selected sample of African maize germplasm under different disease levels and rainfall regimes.

8.2 Materials and Methods

8.2.1 Maize germplasm

Maize inbred lines were obtained from the CIMMYT programme in Harare, Zimbabwe, while the N3 inbred was obtained from the Crop Breeding Institute in Zimbabwe. The inbred lines were sampled from the major heterotic groups that are adapted to subtropical environments and are indicated in Table 8.1. Standard hybrid checks were selected based on their grain yield performance, stability in various environments and reaction to a number of foliar diseases such as phaeosphaeria leaf spot (PLS), GLS, NLB and common rust. Hybrids that are commonly grown by resource-poor smallholder farmers were also included. The environments selected included mainly the mid-altitude tropical maize mega-environments.

Table 8.1 Designation, pedigrees and heterotic groups for parent inbred lines used in the diallel analysis

Designation†	Pedigree or Population (OPVs)	Heterotic grouping
A1220-4	[(CML395/CML444)-B-4-1-3-1-B/CML395//SC/ZM605#b-19-2-X]-1-2-X-1-1-BBBBBB]-7-1-3-2-BBB	B / SC
CML205	[EMSR]#B#bF101sr-2-1-sr-3-2-4-b-b	B
A16	Original pedigree CML312 (S89500F2-2-2-1-1-B*5)	A
CML445	[[TUXPSEQ]C1F2/P49-SR]F2-45-7-5-1-BBB	AB
CML488	DTPWC8F31-4-2-1-5-BBB	AB
CZL00001	INTA-191-2-1-2-BBBB	A
CZL00009	INTA-F2-192-2-1-1-1-BBBBB	A
MP18	[[[NAW5867/P30SR]-111-2/[NAW5867/P30SR]-25-1]-9-2-3-B-2-B/CML388]-B-35-2-B-1-#-1-BB	A / P
N3	Salisbury White	N3
CML443	[AC8342/IKENNE{1}8149SR//PL9A]C1F1-500-4-X-1-1-BB-1-BB	AB

†some of the lines like A1220-4, A16 and MP18 were coded for convenience of study.

8.2.2 Field Evaluations and Diallel Crosses

The ten advanced maize inbred lines were crossed in all possible combinations in a half-diallel mating scheme (excluding selfs). The resulting 45 F_1 single cross hybrids plus nine standard checks were evaluated in a total of eleven test environments (year-location combinations) during 2007/8 and 2008/9 seasons. The environments used and their characteristics are described in Table 8.2.

The F_1 hybrids and standard hybrid checks were laid out in the field in two replications using a 9 x 6 alpha (0, 1) lattice design in each environment. The plot size in each environment was two rows, 3 m long, with 0.75 m inter-row spacing and 0.3 m intra-row spacing, except for Mpongwe were plots where one row, 5 m long with 0.75 m between rows and 0.3 m between the plants. Plant population densities were about 44 000 per hectare in all the environments. Fertiliser was applied at the rate of 120 kg N, 33 kg P, and 44 kg K. Standard cultural practices including ploughing and disking, hand planting, hand weeding and/or application of herbicides and fertilizers were followed at each site. At harvest grain yield was measured on a whole plot basis following standard practice used at CIMMYT (CIMMYT, 1985) and adjusted to 12.5% moisture using the formula:

Grain Yield (t ha^{-1}) = [Grain Weight (kg/plot) x 10 x (100-MC)/ (100-12.5)/ (Plot Area)], where MC = Grain Moisture Content.

Table 8.2 Locations and environments used for evaluations of the F_1 experimental hybrids between 2007/8 and 2008/9 seasons

Location	Country	Year	Code	Latitude	Longitude	Altitude (m.a.s.l)	Rainfall[1] (mm)	Type of Stress
Cedara	South Africa	2007/8 (1)	C108	29°32'S	30°16'E	1130	729	NLB, PLS, common rust, GLS
Cedara	South Africa	2007/8 (2)	C208	29°32'S	30°16'E	1130	301	drought, NLB, PLS
Cedara	South Africa	2008/9	C09	29°32'S	30°16'E	1130	603	NLB, PLS, GLS
RARS	Zimbabwe	2007/8	RA08	17°40'S	31°14'E	1300	806	NLB, MSV, PLS, GLS
RARS	Zimbabwe	2008/9	RA09	17°40'S	31°14'E	1300	846	NLB, MSV, PLS, GLS
Mpongwe	Zambia	2007/8	ZAM08	13°31'S	28°8'E	1219	950	NLB, NCLS[2], PLS, GLS, MSV
Mpongwe	Zambia	2008/9	ZAM09	13°31'S	28°8'E	1219	1100	NLB, NCLS, MSV
Namulonge	Uganda	2007/8	UG08	0°31'N	32°35'E	1150	512	NLB, MSV, GLS
Namulonge	Uganda	2008/9	UG09	0°31'N	32°35'E	1150	327	Drought, MSV, PLS
Baynefield Estate	South Africa	2008/9	BF09	29°46'S	30°21'E	750	844	GLS
KRC	Zimbabwe	2008/9	KD09	18°19'S	29°17'E	1149	763	drought

[1]rainfall refers to the amount received during the growing period, [2]NCLS = Northern corn leaf spot disease caused by *Cochliobolus carbonum R.R. Nelson (anamorph: Bipolaris zeicola (G.L. Stout) Shoemaker*.

8.3 Data analysis

Data from individual and combined seasons were analysed using PROC GLM procedure in SAS 9.1 (SAS Institute, 2002). The data were subjected to ANOVA firstly by environment with genotypes as the main effect, then a combined analysis across environments was conducted to analyse the effect of years, genotypes and interactions. Genotype means were ranked and compared using the t- test (P=0.05). Pearson correlation coefficients were calculated for the environments using the SAS procedure, PROC CORR (SAS Institute, 2002).

8.3.1 Model for AMMI

A total of 11 test environments (year-location combinations) were used for the analysis. Additive Main Effects and Multiplicative Interaction analysis (Gauch, 1992) was performed using the AMMI macros in Genstat 12 (Payne *et al*., 2009).

The following AMMI model was used for the 54 hybrids and 11 test environments (Gauch 1992):

$Y_{ij} = \mu + g_i + e_j + {}^{n'}\Sigma_{n=1}\, \lambda_n \alpha_{in} \gamma_{jn} + \theta_{ij}$

$\theta_{ij} \sim N(0, \sigma^2)$; i = 1, 2, ..., 54; j = 1, 2, ..., 11,

Where Y_{ij} = yield mean of i^{th} hybrid in j^{th} environment; μ = grand mean; g_i = main effects of hybrids; e_j = main effects of environments; λ_n = eigen values for PCA axis n; α_{in} and γ_{jn} = the i^{th} hybrid j^{th} environment PCA scores for the PCA axis n; θ_{ij} is the residual; n' = the number of PCA axes retained in the model.

A full model (AMMIF) was fitted, where all the IPCAs were significant. However, the best AMMI model from AMMIF was selected as suggested by Gauch (1992) by selecting the model that gave less noise. Gauch (1992) defined noise as the difference between a yield estimate and its true mean. The following equation (Gauch, 1992) was used to estimate the percent level of noise in the GE interaction component:

(100 x (Interaction DF x EMS))/Interaction SS
Where*: interaction DF = interaction degrees of freedom,*
EMS = expected error mean square for the AMMI ANOVA,
Interaction SS = interaction sum of squares.

The number of IPCAs in the final model selected was that where the residual sum of squares value was either equal or close to the corresponding sum of squares for the estimated level of noise (Table 8.8).

AMMI biplot was done for AMMI1, which is IPCA1 plotted against genotype and environment means. Hybrids with IPCA scores close to zero were stable, with wide adaptation and those with large IPCA scores (significantly greater than zero) were specifically adapted to the environments that had similar large IPCA scores with the same sign (Crossa *et al*., 1990; 1991).

8.3.2 Model for GGE biplot

In this study GGE biplot was performed using Genstat 12 (Payne *et al*., 2009). The GGE biplot based on the PCA of environment-centred data was used to provide a visual relation among hybrids and test environments (Yan *et al*., 2000, Yan and Hunt, 2002). The following

model for GGE biplot based on singular value decomposition (SVD) of first two principal components (Yan, 2002) was used:

$Y_{ij} - \mu - \beta_j = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_i 2 \eta_{j2} + \varepsilon_{ij}$

Where*: Y_{ij} = yield mean of i^{th} hybrid in j^{th} environment, μ = grand mean; β_j = main effect of environment j; $\mu + \beta_j$ = mean yield across all hybrids in environment j, λ_1 and λ_2 = singular values (SV) for the first and second principal component (PC1 and PC2), respectively, ξ_{i1} and ξ_{i2} = eigen vectors of hybrid i for PC1 and PC2, respectively, $\eta 1j$ and $\eta 2j$ = eigen vectors of environment j for PC1 and PC2, respectively ε_{ij} = residual associated with hybrid i in environment j.*

According to Yan *et al.* (2000), ideal genotypes, using the GGE biplot would be those that showed high PC1 values (high mean yield) and PC2 values close to zero (more stable). The ideal environment for testing should have a high PC1 value that is, it provides better hybrid discrimination and PC2 values near zero, which is a closer representative of the environment mean (Yan *et al*., 2001). To identify the best genotypes in each environment and groups of environments, a polygon view (Yan, 2002) was drawn by connecting hybrids that were furthest from the biplot origin such that all hybrids were enclosed within the polygon. Perpendicular lines were then drawn to each side of the polygon starting from the biplot origin (Yan, 2002).

The biplot was also used to explore the interrelationships among environments by constructing lines (environment vectors) from the biplot origin to markers for the environments. The environments were divided into two sub-sets based on location. Sites North of latitude 18 were grouped together and those below were grouped together and then analysed for similarities. Another line called the average environment axis (AEC) was also used to show the ranking of the hybrids by their mean yield and stability. The line passed through the biplot origin and another line perpendicular to it was drawn to represent the stability of the genotypes. According to Yan (2002), either direction away from the biplot origin on this axis, indicated greater GEI and reduced stability.

8.4 Results

Analysis of variance done on grain yield across the 11 test environments showed the main effects (hybrids and environment), experimental hybrids, hybrid checks and their interaction with the environment to be highly significant (P<0.001, Table 8.4). The contrast of the grain yield for the experimental hybrids against the checks and its interaction with the environment was not significant (P>0.05). The mean grain yield for the experimental hybrids and checks was 6.74 and 6.79 t ha^{-1}, respectively. Single environment analysis showed the hybrids to be significantly different (P<0.05) in all the environments. The minimum and maximum grain yields for each environment are indicated in Table 8.5. The maximum yields ranged from 6.0 to 15.1 t ha^{-1} and the minimum from 1.6 to 5.9 t ha^{-1}. Environment RA08 had the highest maximum grain yield, whereas C208 and UG09 had the lowest yields (both maximum and minimum).

Table 8.4 Analysis of variance (ANOVA) for grain yield (t ha^{-1}) of hybrids tested across 11 environments between 2007/8 and 2008/9 seasons.

Source	DF	SS	Mean Square	F Value	Pr > F
Environment (Env)	**10**	**4430.66**	**443.07**	**279.62**	**<.0001**
Rep(Env)	**11**	**72.86**	**6.62**	**4.18**	**<.0001**
Entry	**53**	**593.02**	**11.19**	**7.06**	**<.0001**
Experimental hybrids (Exp)	44	454.24	10.32	5.99	<.0001
Hybrid checks (Chks)	8	137.30	17.16	17.7	<.0001
Chks vs Exp	1	4.60	4.60	1.55	0.213
Env*Entry	**530**	**1944.49**	**3.67**	**2.32**	**<.0001**
Env*Exp	440	1687.54	3.84	2.22	<.0001
Env*Chks	80	218.65	2.73	2.82	<.0001
Env*Chks vs Exp	10	36.44	3.64	1.23	0.2666
Error	**583**	**923.78**	**1.58**		
Corrected Total	**1187**	**7964.80**			
Means					
Experimental hybrids		6.74			
Hybrid checks		6.79			

Table 8.5 Ranking of the top 20 hybrids based on ANOVA and the minimum and maximum yield (tha^{-1}) obtained at each of the test environments

RANK	C108	C208	RA08	ZAM08	UG08	C09	BF09	RA09	KD09	ZAM09	UG09	Overall
1	S63	H21	H13	H12	H1	H39	S71	H21	H6	H18	H28	H21
2	H8	H29	H31	H31	H11	H9	H38	S71	H21	H44	H3	H14
3	P067	H14	H39	H10	H45	H14	H19	S63	S63	H14	H29	S63
4	H12	H34	H38	H14	P57	N72	H10	H26	H31	H21	H8	H1
5	H21	H13	H14	H33	H43	H33	H1	H11	H9	H15	H9	H11
6	H10	H11	H4	H26	H18	H13	H2	H2	S71	N72	H13	S71
7	H31	H38	H42	H4	H29	H1	H22	H14	H39	H7	H38	H18
8	H39	H31	H6	H45	H44	H26	H7	H28	H32	H26	H26	H44
9	P27	H6	N72	H6	S63	H40	H11	H37	H14	H3	H1	H38
10	H11	H18	H21	H21	H3	H44	H21	H38	H40	S63	H6	N72
11	H30	H1	H26	P067	H35	S63	H6	H1	H12	H19	H24	H26
12	H4	H40	H1	N72	H16	H28	H23	H40	P067	H43	H27	H13
13	H19	H17	S63	H34	H7	P067	H32	P27	H38	H2	P067	H3
14	H45	H24	S71	S63	H19	H2	H45	H30	P77	H45	S71	H19
15	H14	N72	H17	H19	H4	H10	H35	H12	N72	H1	H2	H12
16	H17	H28	P067	H11	H14	H11	H30	H27	P57	H11	H20	H31
17	H43	H2	H44	H17	H21	H19	H15	H35	H8	H30	H18	H8
18	H44	H8	H16	H18	H5	H3	S51	H13	H15	H17	H4	H9
19	H18	H3	H7	S51	H9	H21	S63	H18	H37	H28	S63	H45
20	H33	H36	H3	S71	H42	H17	H44	H4	H30	H24	H23	H39
Mean	8.38	3.39	6.83	7.61	6.01	9.37	8.37	7.26	6.64	8.05	2.81	6.75
Max	10.84	6.05	15.11	10.97	10.71	11.80	12.11	9.89	10.29	13.71	7.05	8.45
Min	5.39	1.66	3.63	4.96	3.48	3.98	5.94	4.49	3.95	3.06	1.61	5.59
CV (%)	13.38	33.34	27.52	14.63	24.85	13.33	13.89	13.00	14.72	17.54	39.99	18.64
LSD (0.05)	2.15	2.27	3.77	2.23	3.00	2.50	2.33	1.88	1.96	2.83	2.25	0.75
P-value	0.0021	0.0256	0.0094	<.0001	0.0262	0.0003	0.0001	0.0003	<.0001	<.0001	0.2900	<.0001

8.4.1 Hybrid Ranking and Phenotypic correlation coefficients of the Test environments

The ranking of the top 20 hybrids based on the mean grain yields from ANOVA are presented in Table 8.5. The hybrid checks that appeared in the top 20 in at least five environments (env) were S63 (10 env), S71 and P067 (6 env). The other hybrid checks appeared in only one (P77) or two (S51, P57 and P27) environments. The experimental hybrids that appeared in the top 20 in at least five of the environments included H21 (10

env), H14 (9 env) H11 (8 env), H1 and H18 (7 env), N72 (6 env) and H31 (5 env). Correlation of the test environments based on grain yield was done using Pearsons phenotypic correlation coefficients and the results are presented in Table 8.6. The significant (P≤0.05) correlations ranged from 0.19 to 0.40. All the correlations were positive with the exception of C208 and UG09 which were negatively correlated (r = -0.23). Test environments that were positively correlated included: C108 with RARS08, ZAMB08, KAD09 and ZAMB09. C208 was positively correlated with RARS08, ZAMB08 with KAD09; RARS09 with C09 and BF09 and ZAMB09 with UG08, C09, BF09 and RARS09.

Table 8.6 Pearson correlation coefficients for environments based on grain yield (t ha^{-1})

	C108	C208	RA08	ZAM08	UG08	C09	BF09	RA09	KD09	ZAM09	UG09
C108	1.00										
C208	-0.03	1.00									
RA08	0.22**	0.25**	1.00								
ZAM08	0.40***	-0.02	0.16	1.00							
UG08	0.10	0.08	0.02	0.07	1.00						
C09	0.18	0.16	0.04	0.12	0.05	1.00					
BF09	0.03	-0.04	0.02	0.06	0.06	0.08	1.00				
RA09	0.13	0.17	0.16	-0.07	0.00	0.22**	0.23**	1.00			
KD09	0.19*	0.04	0.12	0.24**	0.01	0.00	0.02	0.17	1.00		
ZAM09	0.25**	0.15	0.01	0.21	0.23**	0.36***	0.23**	0.37***	0.04	1.00	
UG09	-0.13	-0.23**	0.03	-0.09	0.14	0.21*	-0.03	0.09	0.09	0.18	1.00

*, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001, respectively

8.4.2 AMMI analysis results

Results of the AMMI analysis of variance in 11 test environments on grain yield are presented in Table 8.7. A full model with seven IPCAs was fitted and the IPCA1 to IPCA6 were all highly significant (P<0.001). The hybrids, environments and interaction were all highly significant (P<.001) for grain yield. However, after estimating the contribution of noise and pattern to the interaction sum of squares (Table 8.7), AMMI3 was chosen (with three IPCAs).

Table 8.7 ANOVA for AMMI full model for grain yield (t ha^{-1}) of hybrids tested across 11 environments between 2007/8 and 2008/9 seasons

Source	DF	SS	MS	% Total SS explained	% Treatment SS explained	% Interaction SS explained
Treatments	593	6968	11.75***	87.5	-	-
Hybrids	53	593	11.19***	7.4	8.5	-
Environments	10	4430	443.04***	55.6	63.6	-
Block	11	73	6.63***	-		
Interactions	530	1944	3.67***	24.4	27.9	-
IPCA1	62	616	9.94***	-	-	31.6
IPCA2	60	287	4.78***	-	-	14.7
IPCA3	58	248	4.27***	-	-	12.8
IPCA4	56	193	3.45***	-	-	9.9
IPCA5	54	189	3.5***	-	-	9.7
IPCA6	52	148	2.85***	-	-	7.6
IPCA7	50	88	1.76ns	-	-	-
Interaction residuals	138	175	1.27			
Error	583	924	1.58	12.5		
Total	1187	7965	6.71			

*, **, *** indicates the term is significant at P≤0.05, P≤0.01 and P≤0.001, respectively, ns = not significant

The model (hybrids, environments and interactions) captured 87.5% of the total sum of squares using about 50% of the total degrees of freedom. Of the model (treatment) sum of squares, the hybrids contributed 8.5%, the environments 63.6% and the interaction 27.9%. For the interactions, IPCA1 explained 31.6% of the interaction variation from 11.6% of the degrees of freedom. When IPCA2 was fitted, the first two IPCAs explained 46.3% of the interaction using 23% of the interaction degrees of freedom. Addition of the third IPCA resulted in the first three IPCAs explaining 58.1% of the interaction from 33.9% of the total interaction degrees of freedom.

Results of the contribution of noise and pattern in yield interaction sum of squares are indicated in Table 8.8. After calculating the percentage level of noise in the AMMI full model ANOVA, the best model selected included the first three IPCAs. The level of noise was 43% with an equivalent sum of squares of 837.9. The interaction residual sum of squares for each AMMI model fitted was compared with the noise sum of squares. AMMI3 model was chosen as its residual sum of squares was closest to that from the noise.

Table 8.8 Levels of noise and pattern in yield interaction sum of squares and contributions from each AMMI Model fitted

	% Level	Sum of Squares
Noise	43.1	837.9
Pattern	56.9	1106.1
AMMI Model Fitted		
		Residual Sum of Squares
AMMI1		1328
AMMI2		1041
AMMI3		794
AMMI4		601
AMMI5		411

8.4.3 Best four hybrid selections from AMMI per environment

AMMI gave the best four selections from each test environment and these are presented in Table 8.9. The hybrid which appeared in the top four in at least three environments were; H21 (seven env), H14 (six env), H1 (five env), H11 and H13 (four env), H31 and S63 (three env). The other hybrids; H6, H12, H18, H28, H29, H38, N72 and S71 appeared either twice or once.

Table 8.9: Ranking of the first four AMMI selections per environment

Environment	Season	ENV Code	Mean (tha^{-1})	PCA Score	Rank			
					1	2	3	4
RARS	2007/8	RA08	6.827	1.6085	H13	H31	H39	H14
KRC	2008/9	KD09	6.642	1.1074	H6	H31	S71	H21
Cedara	2007/8 (2)	C208	3.393	0.5082	H13	H1	H14	H21
Mpongwe	2007/8	ZAM08	7.601	0.4396	H31	H12	H21	H14
Namulonge	2008/9	UG09	2.811	0.3383	H1	H13	H11	H38
Cedara	2007/8 (1)	C108	8.004	0.311	H21	H14	S63	H12
Baynesfield Estate	2008/9	BF09	8.375	0.014	S71	H11	H21	S63
RARS	2008/9	RA09	7.206	-0.0985	H1	H21	H11	S63
Namulonge	2007/8	UG08	6.013	-0.2465	H1	H11	H28	H29
Cedara	2008/9	C09	9.365	-0.3978	H13	H14	H1	H21
Mpongwe	2008/9	ZAM09	8.05	-3.5842	H18	H44	H14	N72

8.4.4 AMMI Biplots: Classification of hybrids and environments

The first IPCA was plotted against the means for both the genotype and environment (Fig 8.1). There was a cluster of hybrids both below and above the mean grain yield which had IPCA1 values close to zero (between 0 and +0.5 or -0.5). Some of these hybrids included H22, S51, P57, H29, H16, H10, H8, H17 S63, H21, H14, N72, H24, H30 and H40. Environments RA08, KD09 had large IPCA1 values and had mean yields equal to the overall mean. Hybrids that had the same sign and IPCA values close to these environments were H6, H31, H32, and H13. Environments ZAM08 and C108 were classified above the mean grain yield of all environments and hybrids that had the same IPCA sign and values close to these environments were P067, H39, H38 and H4. Test environments, BF09, RA09 and UG08 had IPCA1 values close to zero, with yields for BF09 and RA09 above the average yield and UG08 below the average yield of all the environments. Environments UG09 and C208 had the lowest mean yield environments and IPCA1 values close to 0.5. ZAM09 had a large negative IPCA1 value and mean yield above 8.0 t ha^{-1}. Hybrids which had negative IPCA values and above average yields included H44 and H18 (IPCA values around -1), H26, N72, H11, H14, H21, S63 (IPCA values close to zero, that is, between 0 and -0.5).

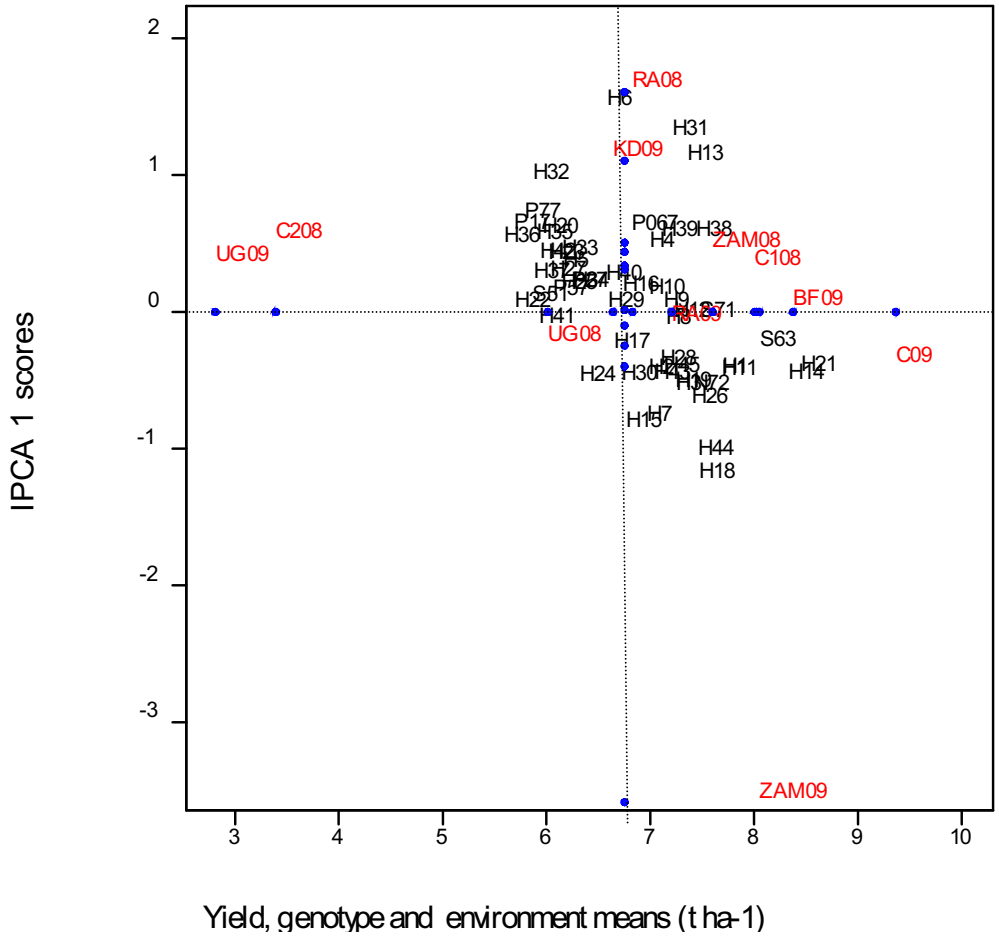

Figure 8.1 AMMI biplot of IPCA1 scores against grain yield (t ha^{-1}) for 11 environments. The environments are: C108 = .Cedara, 2007/8, first planting; C208 = Cedara, 2007/8, second planting; C09 = Cedara, 2008/9; RA08 = Rattray Arnold Research Station (RARS), 2007/8; RA09 = RARS, 2008/9; ZAM08 = Mpongwe 2007/8; ZAM09 = Mpongwe. 2008/9; UG08 = Namulonge 2007/8, UG09 = Namulonge 2008/9; BF09 = Baynesfield, 2008/9; KD09 = Kadoma Research Centre (KRC), 2008/9.

8.4.5 GGE Biplots

From the GGE biplots the first two PCs explained 53.3% (PC1 = 36.2% and PC2 = 17.1%) of the total GGE variation. Results of the different GGE biplots are presented in Figs. 8.2-8.6.

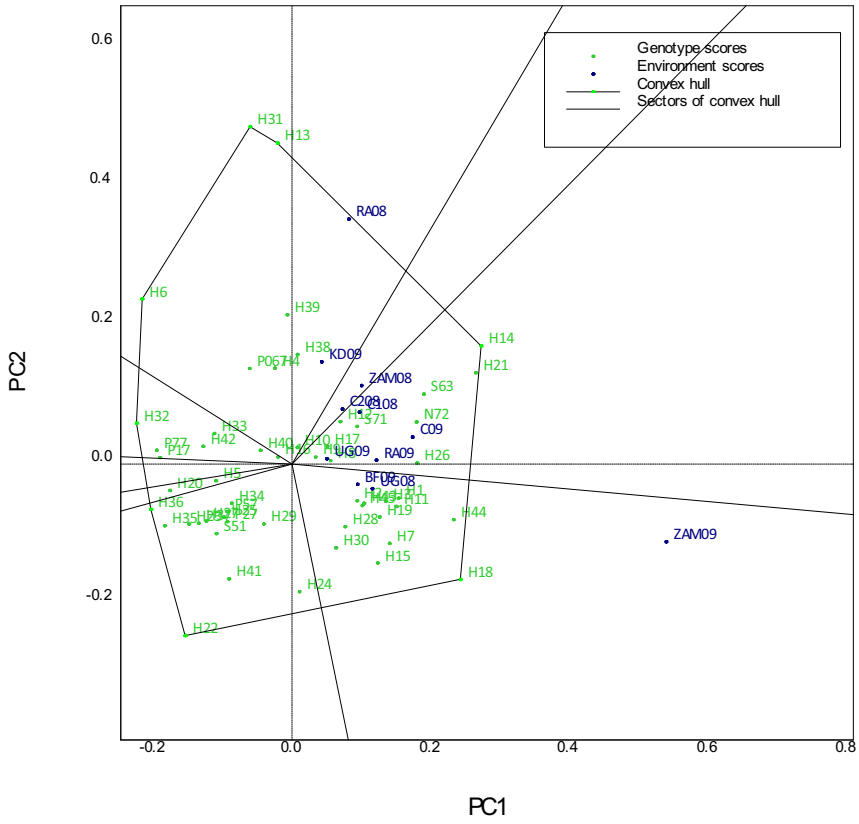

Figure 8.2 Polygon view of the GGE biplot based on grain yield (t ha^{-1}) for 11 environments. The environments are described in Fig 8.1.

8.4.6 Identification of the best hybrids for each environment

The polygon view of the GGE biplot is presented in Fig. 8.2. This biplot indicates the best performing hybrid(s) for each environment and the groups of environments (Yan and Hunt, 2002). The rays of the biplot divided the plot into eight sections. The environments appeared in four of them. The vertex families for each quadrant represented the hybrids with the highest yield for the environment that fell within it. The highest yielding hybrids in environment RA08 and KD09 were H31 and H13; whereas in C208, ZAM08, C108, C09, UG09 and RA09 they were H14 and H21. The highest yielding hybrids in BF09, UG08 and ZAM09 were H18 and H44. The rest of the vertex hybrids H22, H36, H32 and H6 were poor

in the test environments. The rest of the hybrids were located within the polygon and most of them near the plot origin.

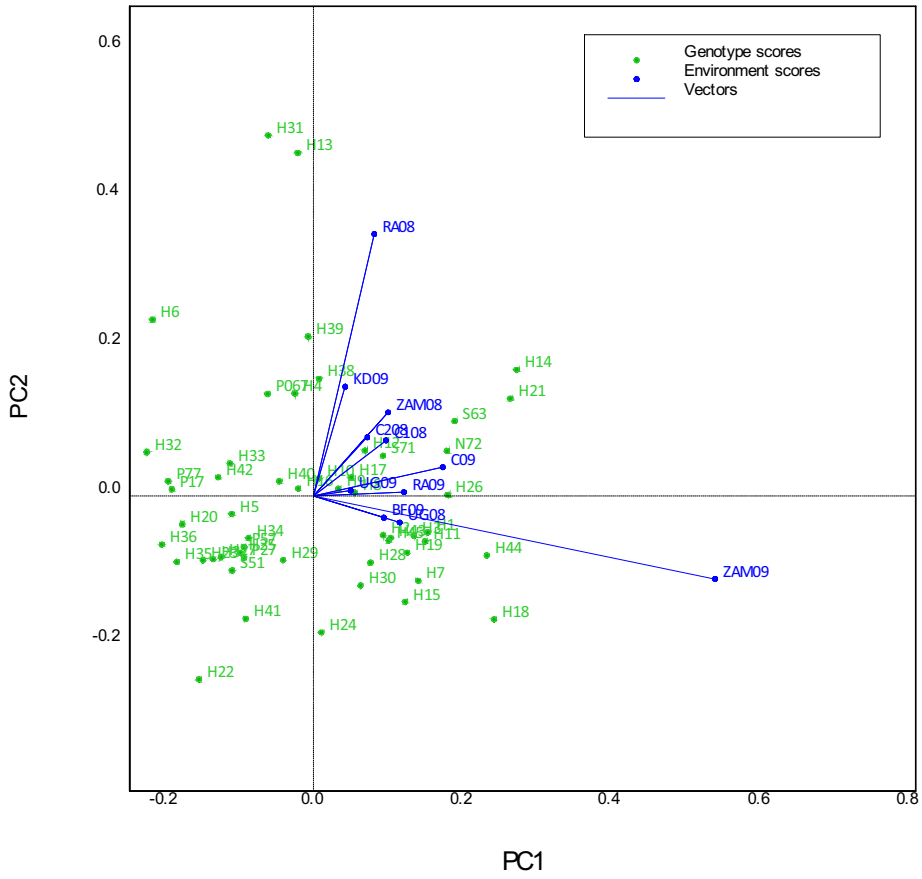

Figure 8.3 GGE biplot based on grain yield (t ha^{-1}) for 11 environments showing the relationship among the environments. The environments are described in Fig 8.1.

8.4.7 Interrelationship among environments

Environment vectors were drawn from the biplot origin to connect the environments (Fig 8.3). The angle between the vectors of two environments was related to the correlation between them. Smaller angles less than 90° showing high correlations were observed for most of the environments. The angle between RA08 and ZAM09 was almost 90° and that between RA08 and BF09 and UG08 was slightly more than 90°. The highest correlations were between: R08 and KD09; ZAM08, C208 and C108; C09, UG09 and RA09 and BF09, UG08

and ZAM09. Most of the environments had short vectors and PC2 values close to zero. The longest vectors were observed for RA08 and ZAM09.

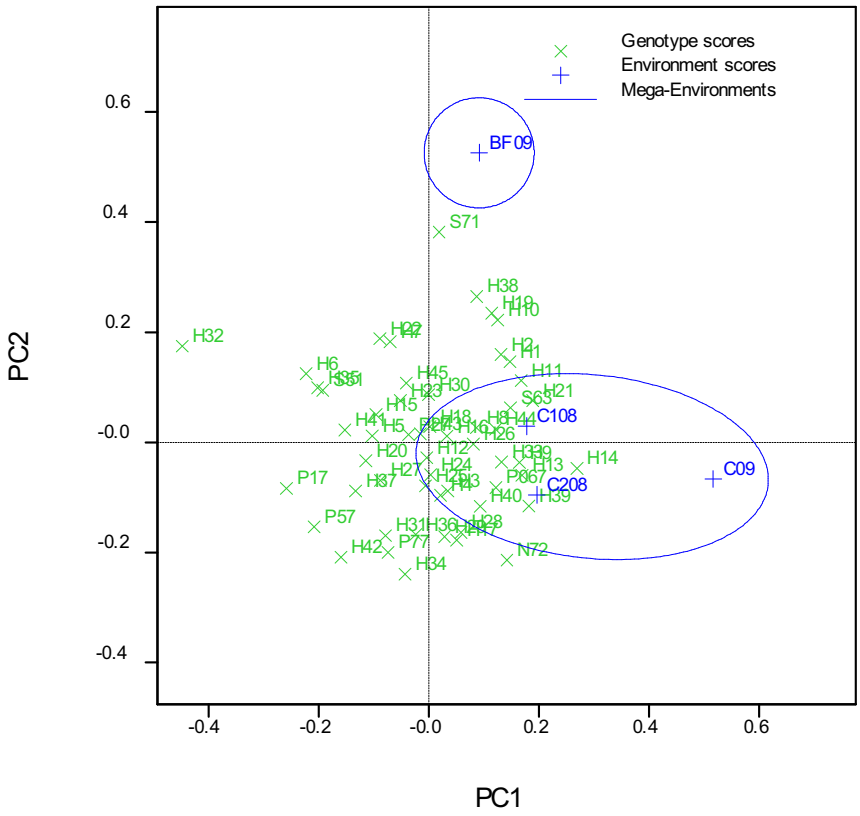

Figure 8.4 GGE biplot based on grain yield (t ha^{-1}) for four environments south of latitude 18, showing the relationship among the environments. The environments are described in Fig 8.1.

The two sites south of Lat18 showed differences (Fig 8.4). The three Cedara environments were grouped together, whereas the Baynesfield site was on its own. Baynesfield location had a PC1 score close to the origin, whereas the Cedara environments (C108 and C208) had a PC1 score of 0.2 and C09 a PC1 score close to 0.6. Cedara environments had PC2 scores close to the origin, whereas Baynesfield location had a positive PC2 score close to 0.6.

The sites located north of Lat18 had some overlaps (Fig. 5). All the environments with the exception of ZAM09 (PC score close to 0.6), had PC1 scores close to the origin (below 0.2). All the environments with the exception of RA08 had PC2 scores close to the origin (below 0.2). RA08 had a positive PC2 score close to 0.4.

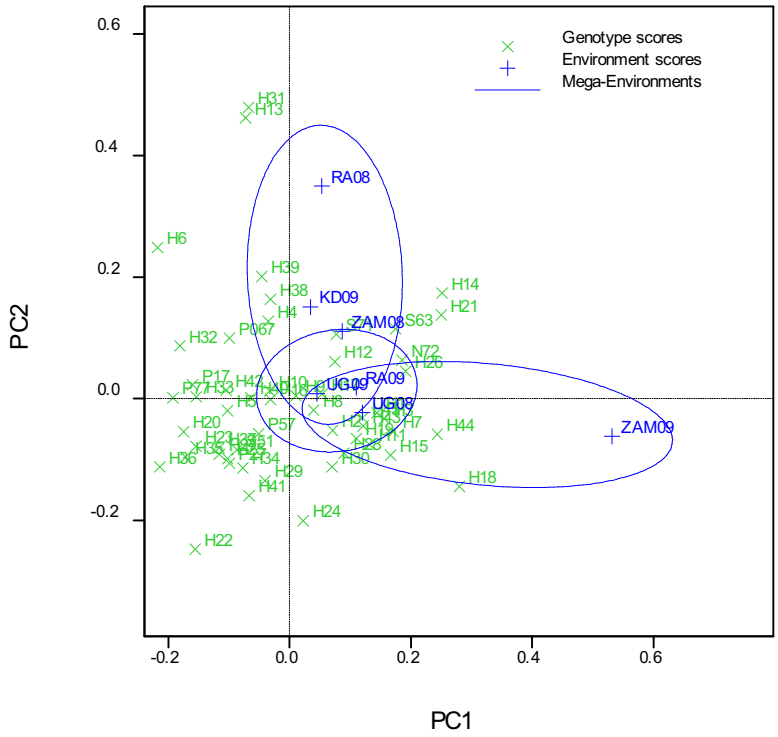

Figure 8.5 GGE biplot based on grain yield (t ha^{-1}) for 7 environments, north of latitude 18 showing the relationship among the environments. The environments are described in Fig 8.1.

8.4.8 Ranking of hybrids based on mean yield and stability

The hybrids that were close to the origin and had the shortest vectors from the AEC line were defined as high yielding, high stability hybrids (Fig. 8.6). The high yield and high stability group comprised H14 (A1220-4 x A16), H21 (CZL00009 x A16), S63 (SeedCo hybrid check), N72 (MP72/N3), H12 (A1220-4 x N3) and H26 (CZL00001 x A16). These hybrids had projections onto the AEC close to zero. They were close to the origin and had shortest

vectors from the AEC. Hybrids H22 (CZL00009 x MP18), H41 (MP18 x CML488), H31 (N3 x A16), and H13 (A1220-4 x CML205) were the worst in terms yield and stability, they had longest vectors from the AEC and PC1 values below zero. Hybrids H44 (CZL00009 x CML443) and H18 (CZL00009 x CZL00001) (for ZAM09) had long vectors from the AEC line but high PC1 scores and H1 (CML445 x A1220-4), and H11 (A1220-4 x CZL00001) for BF09 and UG08.

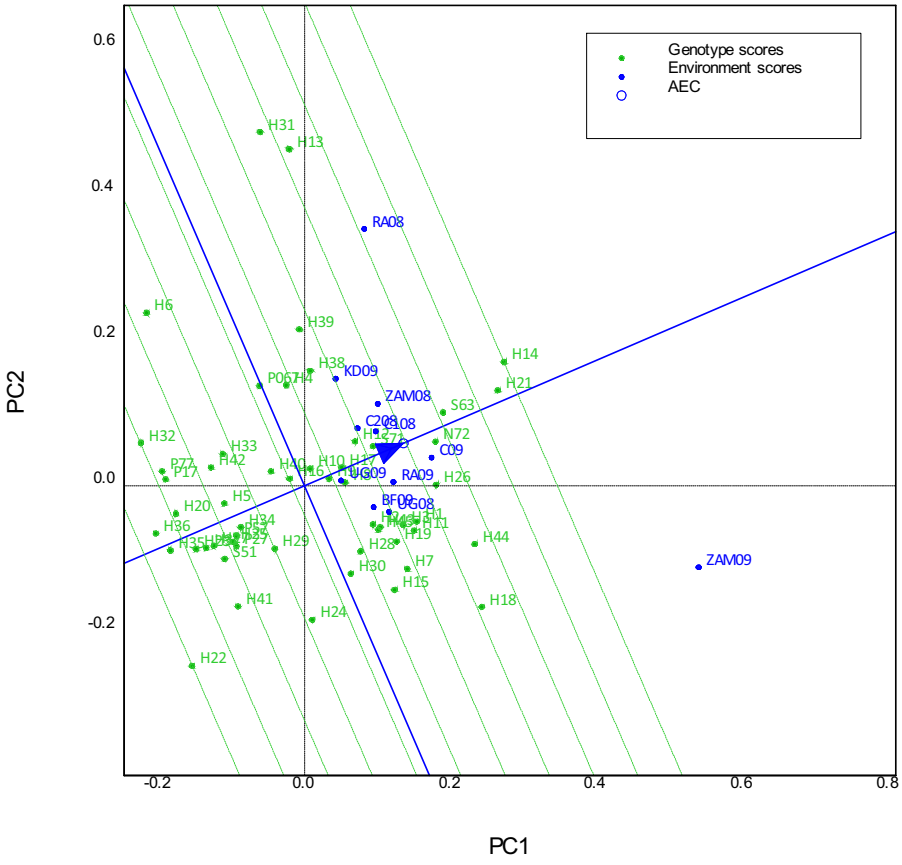

Figure 8.6 GGE biplot based on grain yield (t ha^{-1}) for 11 environments ranking hybrids based on both the mean grain yield (t ha^{-1}) and stability. The environments are described in Fig 8.1.

8.5 Discussion

The significant hybrid main effects and hybrid x environment interactions for grain yield indicated that the hybrids were different and the environments diverse. However, the experimental hybrids and the hybrid checks were similar in their performance across the environments. From the AMMI analysis, it was observed that the environment had the greatest effect, accounting for almost 64% of the variation in the treatments. The genotypes had the least effect accounting for about 9% showing a lower variability among the hybrids, whereas the GEI accounting for about 27% of the treatment variation. The first two IPCA scores explained 46.3% of the interaction sum of squares. The highly significant effects of the environment indicated high differential hybrid responses across the different environments. Variations in the rainfall amounts and distributions plus other stresses such as diseases could have contributed immensely to these differences. For example, C208 and UG09 both experienced serious mid-season droughts during the growing season and C208 had high levels of PLS disease. The average yields were highly variable ranging from 2.8 to 9.3 t ha^{-1} across the environments.

8.5.1 Ranking of hybrids

The ranking of the top four hybrids based on AMMI analysis was different from the ranking of the unadjusted means from the ordinary ANOVA procedure. Only in three environments (RA08, KD09 and ZAM09) out of the 11 did AMMI and ANOVA give the same three hybrids in the top four, but not in the same order. In five of the environments (RA08, UG08, BF09, KD09 and ZAM09), AMMI and ANOVA had the same hybrid in the first position. The difference between the hybrid rankings based on these two methods could be due to the level of noise that was detected. AMMI indicated that 56.9% of the interaction sum of squares was due to pattern, while 43.1% was random or noise variation. The level of noise in the unadjusted means could have elevated some of the hybrids to higher positions resulting in the differences observed with AMMI rankings. A best model is considered to capture the pattern and reject the noise contained in the data (Gauch, 1988). Other researchers have also shown that for different crops, AMMI estimates rank top performing

genotypes differently in almost 50% of the environments when compared with unadjusted means (Dixon and Nukenine, 1977; Crossa *et al*., 1990; Aina *et al*., 2007). More precise estimates are thus required in order to increase the probability of making successful selections. It appears therefore that the differences observed in this study in the rankings between AMMI and the unadjusted means were caused by the random variation or noise. AMMI has been shown to partition a noise-rich residual from the interaction degrees of freedom, while the error control is achieved by discarding the residual (Aina *et al*., 2007). The level of noise observed in this study was comparable to the 30-45% reported by other researchers (Gauch, 1992; Ebdon and Gauch, 2002).

8.5.2 Correlation between test environments

According to Fox *et al*. (1997), environments can be positively correlated or considered alike for selection purposes regardless of their yield, if they rank genotypes similarly. Pearson phenotypic correlation coefficients in this study showed some positive, but weak correlations amongst a number of environments, implying that these environments provided similar differentiation or ranking of a number of hybrids. Environments that were positively correlated included: C108 with RA08; ZAM08, KD09 and ZAM09 with RA08; ZAM08 with KD09; RA09 with C09 and BF09; and ZAM09 with UG08, C09, BF09 and RA09. A few of these positive correlations were confirmed by results from AMMI biplots, although by and large the results differed. For example, AMMI biplots showed environments RA08, KD09 to have large positive IPCA1 scores and mean yields equal to the overall mean, indicating they were positively correlated with each other. Environments ZAM08 and C108 were positively correlated as they were both classified above the mean grain yield of all environments and had positive IPCA1 scores. Test environments, BF09 and RA09 had IPCA1 values close to zero with above average yield. However, UG08 had negative IPCA1 values close to zero and below average yields indicating a negative correlation with BF09 and RA09.

The environments that were negatively correlated with each other based on the Pearson phenotypic correlation coefficients were C208 and UG09. This implied that, hybrids that were high yielding in one of these two environments were low yielding in the other

environment. However, according to AMMI biplot, these two environments UG09 and C208 had the lowest mean yield and positive IPCA1 values close to 0.5, showing a positive correlation between them. Positive correlations indicated that the environments provide similar discrimination of genotypes.

8.5.3 AMMI Biplots: Classification of hybrids and environments

There was a cluster of hybrids both below and above the mean grain yield which had IPCA1 values close to zero. Hybrids with IPCA1 scores close to zero and near the origin showed little interaction with environments. In other words they are less responsive to environmental changes. Some of these hybrids included H22, H41, H29, H9, H8, H12, H17, H16, H10 and S51. Other hybrids had above average yields and relatively small IPCAs scores (between 0 and +0.5 or -0.5). These hybrids were relatively stable, showing a wider adaption to test environments. According to Fox *et al*. (1997), a genotype found in the top third of entries across environments can be considered relatively well adapted. In this study this group includes hybrids such as H21 (CZL00009 x A16) and H14 (A1220-4 x A16) which were the highest yielding hybrids and others like S63 (SeedCo hybrid Check), H11 (A1220-4 x CZL00001), N72 (MP72/N3), H38 (A16 x MP18) and H26 (CZL00001 x A16). A large hybrid IPCA1 score reflected a more specific adaptation (unstable) to environments. Hybrids H6 (CML445 x A16), H31 (N3 x A16), and H13 (A1220-4 x CML205), H32 (N3 x MP18), H44 (CZL00009 x CML443), H18 (CZL00009 x CZL00001), H7 (CML445 x MP18), and H15 (A1220-4 x MP18) had high hybrid IPCA1 scores, indicating they were unstable across environments and could be selected for specific adaptation in the test environments they interacted positively with.

The environments that had a high IPCA1 score included RA08 and KD09, implying that these environments were unstable. ZAM09 was another unstable environment and the best performers in that environment were H44 (CZL00009 x CML443), H18 (CZL00009 x CZL00001), which had high IPCA1 scores as well as the same sign as ZAM09. High yielding environments which were relatively stable (that is had IPCA1 scores between 0 and ±0.5) were ZAM08, C108, BF09, RA09 and C09. Stable environments had the least interactions with the hybrids.

8.5.4 GGE Biplots: Identification of the best hybrids for each environment

From the polygon view, the 11 environments appeared only in four of the sectors. According to Yan *et al*. (2007), when different environments fell into different sectors, it implied that they had different high yielding cultivars for those sectors and it showed cross-over GE, suggesting that the test environments could be divided into mega-environments. In this study, the highest yielding hybrids in environment RA08 and KD09 were H31 (N3 x A16), and H13 (A1220-4 x CML205). This result was also in agreement with the observation from the AMMI biplot for these two environments. For C208, ZAM08, C108, C09, UG09 and RA09, the highest performing hybrids were H14 (A1220-4 x A16) and H21 (CZL00009 x A16). These two hybrids were found in AMMI analysis to be high yielding and relatively stable across a number of environments. The two hybrids were close to the ideal hybrids. Yan and Tinker (2005) described the ideal hybrids as having high yield (large PC1 score) and stable across environments (PC2 close to zero). The highest yielding hybrids in BF09, UG08 and ZAM09 were H44 (CZL00009 x CML443), H18 (CZL00009 x CZL00001). However, according to the AMMI biplot, these two hybrids were unstable (had large IPCA1 scores). The rest of the vertex hybrids H22 (CZL00009 x MP18), H36 (CML205 x CML443), H32 (N3 x MP18) and H6 (CML445 x A16) were poor in the test environments. This means that they were not the highest yielding in any of the test environments. The rest of the hybrids were located within the polygon and most of them near the plot origin. According to Yan *et al*. 2001, genotypes within the polygon, especially those located near the plot origin, were less responsive than the vertex genotypes (Yan *et al*., 2001).

8.5.5 Interrelationship among environments

Environments with the longest vectors from the biplot origin were the most discriminating of the hybrids. However, RA08 despite having a long vector had a high PC2 score and a low PC1 score (closer to zero) implying that it was unstable and low yielding. This environment had average yield, despite having the maximum hybrid yield. In addition, it had the largest LSD, suggesting it was one of the worst environments. ZAM09 was the other test environment with a long vector. However, it had a lower PC2 score, indicating relatively stable and high PC1 score indicating high yield. The most representative environments were

KD09, ZAM08, C208, C108, UG 09, C09 and RA09 as they had PC1 and PC2 scores close to zero, indicating stability and high yield. The angle between the vectors of two environments was related to the correlation between them. According to Krooneberg (1995) and Yan (2002), the cosine of the angle between the two vectors of two environments approximated the correlation coefficient between them. Based on these angles, test environments in this study were divided into four main groups but with overlaps. Smaller angles less than 90° showed high correlations. These were observed for most of the environments which included R08 and KD09; ZAM08, C208 and C108; C09, UG09 and RA09, and BF09, UG08 and ZAM09.

Sub-dividing the environments based on their locations revealed that the Cedara environments were different from the BF09 environment. Although all the three environments had above average yields, the BF09 environment had high GLS disease pressure, whereas the Cedara environments (C108, C208 and C09) had high PLS, GLS and NLB disease pressure. The environments north of latitude 18 had some overlaps indicating they were similar to each other. In this group, RA08 and ZAM09 behaved differently from the other environments. The environment RA08 was again confirmed to be unstable and low yielding based on the high PC2 score and a low PC1 score (closer to zero), respectively. The ZAM09, on the other had a lower PC2 score, indicating it was relatively stable and a high PC1 score indicating high yield.

Most of these environments were also positively correlated in both AMMI analysis and Pearson phenotypic correlation coefficients. The angle between RA08 and ZAM09 was almost 90° implying that there was no correlation between the two environments. The hybrids in either environment responded differently. The angles between RA08 and BF09; RA08 and UG08 were slightly more than 90°. An angle greater than 90° meant a negative correlation.

8.5.6 Ranking of hybrids based on mean yield and stability

Hybrids that were stable were located either on the AEC abscissa (Fig 8.4) and had a near zero projection onto the AEC ordinate (Yan *et al*., 2001). In this study the hybrids that were

close to the origin and had shortest vectors from the AEC line were defined as high yielding and stable hybrids. This group comprised H14 (A1220-4 x A16), H21 (CZL00009 x A16), S63 (SeedCo hybrid check), N72 (MP72/N3), H12 (A1220-4 x N3) and H26 (CZL00001 x A16). It means that these are ideal hybrids that can be used for broad selection as they had a high consistent ranking across the environments. Hybrids H22 (CZL00009 x MP18), H41 (MP18 x CML488), H31 (N3 x A16), and H13 (A1220-4 x CML205) were the worst, they had longest vectors from the AEC and PC1 values below zero. Hybrids H44 (CZL00009 x CML443) and H18 (CZL00009 x CZL00001) (for ZAM09) had long vectors from the AEC line but high PC1 scores and these were ideal for specific selection for the ZAM09 environment as they had high yield but low stability. It implied that they responded best to this ZAM09 test environment. Hybrids H1 (CML445 x A1220-4), and H11 (A1220-4 x CZL00001) responded best to the BF09 and UG08 environments.

8.6 Conclusion

Both AMMI and GGE analyses were able to show the best genotypes that had wide adaptation. AMMI analysis identified H21 (CZL00009 x A16), and H14 (A1220-4 x A16), S63 (SeedCo hybrid check), H11 (A1220-4 x CZL00001), N72 (MP72/N3), H38 (A16 x MP18) and H26 as having wider adaptation and were amongst the high yielding hybrids. This selection coincided with the GGE biplot selection, which selected H14 (A1220-4 x A16), H21 (CZL00009 x A16), S63 (SeedCo hybrid check), N72 (MP72/N3), H12 (A1220-4 x N3) and H26 (CZL00001 x A16) as the ideal genotypes, which were high yielding and stable across environments. Hybrids identified as unstable and suitable for specific adaptation were H6 (CML445 x A16), H31 (N3 x A16), H13 (A1220-4 x CML205), H32 (N3 x MP18), H44 (CZL00009 x CML443), (CZL00009 x CZL00001), H7 (CML445 x MP18), and H15 (A1220-4 x CML443) in AMMI analysis and in GGE biplots these were H44 (CZL00009 x CML443) and H18 (CZL00009 x CZL00001), H1 (CML445 x A1220-4), and H11 (A1220-4 x CZL00001), which were unstable but high yielding. The GGE biplots gave more visual interpretations than just selecting the best performing hybrids and it also allowed visualization of crossover GEI through the polygon view. AMMI analysis identified ZAM08, C108, BF09, RA09 and C09 as the high yielding environments which were relatively stable (had IPCA1 scores between 0 and ±0.5). GGE biplots selected KD09, ZAM08, C208, C108, UG09, C09 and RA09 as the

most representative environments (had PC1 and PC2 scores close to zero), indicating stability and high yield. Overall, the AMMI analysis and the GGE biplot analysis resulted in more or less similar selections of superior, stable hybrids and best performing environments.

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9 General Overview

9.1 Introduction

The research focus for this study was to explore resistance sources in the regionally dominant germplasm backgrounds so as to improve resistance to PLS and other important foliar diseases in the important heterotic groups. The first step towards formulating effective breeding strategies was to understand the genetic variability and inheritance of the resistance. In addition, the highly variable environments in sub-Saharan Africa (SSA) make investigation of yield stability an important factor in the breeding programmes. This research focus was used to formulate the objectives of the study and the hypotheses that were to be tested. This chapter therefore gives an overview of the whole study by reiterating the main research hypotheses that were tested. It highlights the main findings of the study, their implications and assists in mapping the way forward for future research.

The following hypotheses were tested:

1. Smallholder farmers are aware of the major constraints that affect maize production in their areas and prefer specific traits and stress tolerance levels in their maize cultivars.
2. Adapted regional maize germplasm has wide genetic variability and possesses high levels of resistance to PLS that can be identified and exploited in breeding programmes.
3. The additive-dominance model is sufficient in explaining maize resistance to PLS and GLS.
4. Maternal effects contribute to the inheritance of resistance to PLS and GLS in maize hybrids.
5. The selected adapted elite tropical maize inbred lines have good combining ability for grain yield and resistance to PLS, GLS, NLB and common rust.
6. Levels of resistance to PLS and grain yield in maize are affected by changes in environment.

9.2 Summary of the major findings

9.2.1 Identification of Farmers' Key Maize Production Constraints and Traits Desired in Maize Cultivars

A survey and participatory rural appraisal (PRA) conducted in Obonjaneni, Busingatha and Okhombe villages in the Northern Drakensberg established that in general farmers' landholdings were small (average 1.4 ha) and maize was the principal crop grown in the area. The highlights of the study were:

- The local variety, *Natal-8-row* or *IsiZulu* was more popular than the hybrids and improved open pollinated varieties (OPVs).
- Farmers preferred the local variety mainly for its taste, and that the seed can be recycled, tolerance to abiotic stresses and yield stability.
- Preferred characteristics of maize varieties in order of importance were: inexpensive seed, high yield, early maturity and low input costs, drought resistance, pest/disease resistance, more rows per cob, taste, prolificacy, wide range of uses, and good for sale.
- Characteristics of an *"ideal"* variety for Amazizi district as listed by the farmers in order of importance were: high yield, good taste, low inputs and inexpensive seed, early maturing, disease resistance and tolerance to acid soils, drought resistance, yield stability, prolificacy, insect resistance, wide range of uses, resistance to lodging and weevils, and good cooking qualities.
- Two improved OPVs Afric1 and Kalahari Early Pearl were ranked amongst the top varieties grown in their area ahead of the hybrids and local variety, based on the characteristics of an ideal variety.
- Abiotic stresses (drought, heavy rains, storms and soil fertility) were amongst the top four constraints faced by the farmers, whereas biotic stresses (weeds, insects and diseases) were ranked fourth, fifth and sixth.
- Stalk borer and cutworms were the most important pests in the area.
- Diseases such as PLS, GLS, NLB, common rust and cob rots, although observed were not a major problem.

9.2.2 Genetic variability of Tropical Maize Germplasm to Phaeosphaeria Leaf Spot Disease under Field Conditions

- A wide range of maize germplasm adapted to tropical and subtropical environments of Africa were evaluated for PLS resistance and monitored for disease progress. Phaeosphaeria leaf spot disease was observed after flowering, and in most of the susceptible genotypes, the disease progression during the season was rapid.
- Significant variation was observed among the inbreds, populations and hybrids with 63% of the inbreds/populations being resistant to PLS.
- Regionally important inbred lines; SC and N3, and CIMMYT's most successful lines such as CML395, CML444, CML206, CML312, and CML488 were resistant.
- Fifty-four percent of the single-cross experimental hybrids and 46% of the commercial hybrids were resistant to PLS.
- There was a significant positive correlation between AUDPC values for disease severity with PLS final disease scores.
- Resistance was moderate to highly heritable (57 to 83%).

9.2.3 Combining Ability Analysis for Phaeosphaeria Leaf Spot and agronomic traits in Tropical Advanced Maize Inbred Lines

Forty five F_1 hybrids generated by crossing ten inbred lines in a half diallel mating scheme were evaluated for combining ability, gene action and heterosis estimates for resistance to PLS.

- General combining ability (GCA) and specific combining ability (SCA) effects were highly significant for PLS, grain yield and other agronomic traits (days to anthesis, days to silking, ear height and plant height).
- The GCA effects accounted for 65 to 90% and SCA effects for 10 to 35% of the variation in the hybrids for PLS resistance, grain yield and the other agronomic traits measured.
- Resistant inbred lines that displayed good combining ability for PLS resistance were A1220-4, N3, A16, MP18 and CML448.
- Parental lines A1220-4, N3, CML205, A16 and CML443 had positive GCA effects for grain yield and contributed towards high yield.
- A1220-4 and A16 were late maturing, whereas CZL00009 displayed early maturity.

- Hybrids with significant negative SCA effects for PLS were CML445 (MR) x N3 (R), A1220-4 (MR) x CZL00009 (S) and CZL00009 (S) x CML488 (R).
- Hybrid A1220-4 (MR) x CZL00009 (S) had the highest negative mid-parent and better-parent heterosis for PLS resistance.

9.2.4 Generation Mean Analysis of Phaeosphaeria Leaf Spot Resistance in Six Tropical Advanced Maize Inbred Lines

Reciprocal crosses and backcross progenies generated among inbreds A1220-4, A15, B17 (resistant, R), CML445 (moderate, MR), CML441 and CZL00001 (susceptible, S) were evaluated for PLS resistance at Cedara Research Station in South Africa. Results were as follows:

- The R x S crosses confirmed that resistance to PLS in these inbreds was predominantly controlled by genes with additive effects.
- Significant dominance and epistasis gene effects were also detected for PLS resistance in the six tropical advanced inbreds
- Cytoplasmic gene effects for PLS resistance were significant especially when the susceptible CML441 was used as female in R x S crosses.
- Transgressive segregation was observed in all groups of crosses (MR x S, S x S and R x R) towards both resistance and susceptibility.
- Frequency distributions for the F_2 and backcross progenies were consistent with quantitative inheritance.
- Mid-parent heterosis values for the R x S crosses were negative indicating heterosis towards resistance.
- The average degree of dominance values in the R x S crosses indicated incomplete dominance.
- Most of the R x S crosses had a minimum of one to four genes conditioning the resistance.

9.2.5 Generation Mean Analysis and Combining Ability for Grey Leaf Spot Resistance in Elite African Maize Germplasm

Forty-five F_1 hybrids generated from a half-diallel mating design were evaluated for combining ability and the types and magnitude of gene action for GLS resistance in tropical elite maize inbreds. Reciprocal and backcross progenies among elite inbreds A1220-4, A15, CML441 (resistant, R), and N3 and B17 (susceptible, S), were evaluated in generation mean analysis (GMA) at Cedara Research Station in South Africa.

- General combining ability (GCA; 71%) and specific combining ability (SCA; 29%) effects were highly significant for GLS resistance.
- The most resistant inbred lines A1220-4, CZL00009, CZL00001, CML205 and CML443 displayed good GCA for GLS resistance.
- The cross N3 (S) x CML205 (R) had the highest amount of heterosis for GLS resistance.
- Generation mean analysis (GMA) showed that additive effects were highly significant and contributed >89% of the total variation due to generations.
- Dominance effects accounted for 7% of the variation in A15 x B17 cross,
- Epistasis was observed in CML441 x N3 and A1220-4 x B17 crosses.
- Reciprocal cross differences were not detected in the F_1 hybrids.
- Resistance was controlled by two to three genes exhibiting zero to partial dominance.
- GLS resistance was moderate to highly heritable (54 to 92%).

9.2.6 Diallel Analysis of Resistance to Northern corn leaf blight and Common Rust diseases in Tropical Advanced Maize Inbred Lines

This study was conducted to determine the combining ability, gene action and heterosis estimates for resistance to NLB and common rust among selected tropical advanced maize inbred lines crossed in a half diallel mating scheme.

- General combining ability (GCA) and specific combining ability (SCA) effects were highly significant for NLB and common rust diseases
- GCA effects accounted for about 74% and SCA effects 26% of the variation in the hybrids for both NLB and common rust resistance
- The resistant inbred lines with good GCA to NLB were A1220-4, CZL00009, CML443 and A16.

- Inbred lines used CML445, A16 and CML443 had good GCA for common rust resistance.
- Lines A16 and CML443 had good GCA for both NLB and common rust resistance.

9.2.7 Genotype-Environment Interaction and Grain Yield Stability of African Maize Germplasm across different Stress Environments

This study was to evaluate the level of grain yield stability and identify the best performing genotypes for wide and specific adaptation in different African environments.

- The first two IPCA scores in AMMI analysis explained 46.3% of the interaction sum of squares.
- In GGE biplot analysis, the first two principal component axes explained 53.3% of the total GGE variation.
- Common hybrids selected by AMMI and GGE biplot as stable and high yielding were: H21 (CZL00009 x A16), H14 (A1220-4 x A16), S63 (SeedCo hybrid check), N72 (MP72/N3) and H26 (CZL00001 x A16).
- Hybrids; H1 (CML445 x A1220-4), H44 (CZL00009 x CML443) and H18 (CZL00009 x CZL00001) were identified by both methods as unstable but high yielding in specific environments.
- AMMI and GGE biplot analyses identified ZAM08, C108, RA09 and C09 as the high yielding and stable environments.

9.3 Implications of the findings in breeding for resistance to diseases and way forward

Results of the PRA established the importance of maize in a smallholder farming sector in South Africa. Farmers preferred varieties that allowed them to save seed rather than buy seed every season. However, high yield was still amongst the top most preferred characteristics of maize in the area. It also confirmed that, despite more land being allocated to maize, yields are still low. From this study, it was evident that the local varieties had high yield potential and genetic variability for disease resistance as indicated by the results from the researcher managed trials. Efforts should therefore be made to address the production

constraints in the area that may be contributing to the low yields; otherwise the high yields will never be realized.

As the farmers indicated, the top most production constraints were abiotic (drought, heavy rains, storms and soil fertility). Some of these constraints can be addressed through breeding and some through good agronomic practices. For example, breeding opportunities for drought tolerant varieties and low nitrogen (N) clearly exist. Varieties adapted to low N can be bred and the characteristics preferred by the farmers such as taste incorporated. Most of the farmers did use fertilizers, but the quantities and types of fertilisers were wrong in most of the cases. Extension support is thus vital in this respect, to assist the farmers in making right, informed decisions in their crop production.

Adoption of hybrids in the area is still low, despite South Africa having many seed companies that produce hybrid seed. Although the farmers indicated their willingness to grow hybrids because of the high yields, they made it clear that the seed and inputs required to grow hybrids were expensive and they also did not like the taste. From the farmers' sentiments, it was evident that seed of any variety that may be introduced in the area has to be less expensive. The way forward is therefore to come up with improved OPVs or synthetics that incorporate the farmers' preferences such as taste, white mealie-meal and resistance to the major abiotic stresses. Procurement of inexpensive seed will remain a challenge, unless government comes up with a lot of incentives especially on the pricing of maize seed and sale of the maize produced, to encourage planting of high yielding maize hybrids in the smallholder sector. As long as the farmers perceive no advantages in growing hybrids they will not adopt them.

Another important trait that the farmers mentioned was early maturity. The farmers are aware that they can evade disease, drought, snow and frost by planting early. This is another opportunity for breeders to breed for early maturing varieties, which can assist the farmers in escaping most of these abiotic and biotic stresses. The biggest challenges though are the frequent occurrences of heavy rains and storms. It is possible to breed for varieties

that can withstand strong winds (lodging resistance) and maybe excessive waterlogging as a result of heavy rains.

Results of the germplasm screening demonstrated that high levels of disease resistance were available in the regionally adapted germplasm. The additional sources of resistance that were identified can be made available to breeding programmes. The experimental hybrids that exhibited high levels of resistance can be recommended for further testing and release.

Symptoms of PLS were noticed around flowering time and there was a negative correlation between flowering days and the final PLS disease severity scores and AUDPC values. This observation actually implies that, depending on the weather conditions and how the disease progresses, the early appearance of the disease has great potential to cause serious reductions in grain yield. A trend similar to this was observed in Brazil where PLS initially did not cause any major damages to the maize quality or grain yield, but with time inoculum started building up over the seasons resulting in significant damage on maize and grain yield reductions of more than 60% in susceptible cultivars (Cervelatti *et al*., 2002; Silva and Moro, 2004). This implies, therefore, that although PLS appears not to be causing any significant yield losses in the region at present, it has the potential of causing serious damage as the inoculum increases. It is imperative, therefore, that PLS resistant varieties be made available to farmers.

The results also revealed more disease in the late planted crop. Therefore early planting should actually be recommended as a mechanism to escape disease. Severe infestations of PLS have been shown to occur in late plantings (Fernandes, 1998; Cervelatti *et al*., 2002).

Positive correlation coefficients between AUDPC values for disease severity with PLS final severity scores implied that ranking of the genotypes for AUDPC and final PLS disease severity score was by and large similar. Therefore a single assessment for the final disease severity would be adequate, especially for screening large numbers of germplasm, than the several assessments required to obtain AUDPC values. As a way forward, only one or two

assessments can be done around flowering and at the hard dough stage which normally reflects the total amount of disease in the season.

Combining ability studies for grain yield and disease resistance identified some lines that had good GCA for resistance to two or more diseases, high grain yield and resulted in high heterosis estimates. Some of these lines were: A1220-4 was good for grain yield and had good resistance to PLS, GLS and NLB; A16 was good for grain yield and resistance to PLS, NLB and common rust; CML443 had good resistance to GLS, NLB, and common rust. The other lines that were identified as resistant sources were: N3 and CML488 for PLS and NLB resistance; MP18 for PLS resistance, CZL00009 and CZL00001 for GLS and NLB resistance; CML205 for GLS resistance and CML445 for common rust resistance. Since most of the symptoms for these diseases were observed simultaneously on the same plant, breeding for multiple disease resistance is therefore recommended for these foliar diseases. This study has identified a few sources that can be used for multiple disease resistance breeding, but more sources would be needed. CZL00009 displayed early maturity and can be used in breeding for early maturing varieties, whereas A1220-4 and A16 had high grain yield potential and are late maturing.

Overall, significant variation was observed among the inbreds adapted to the tropical and subtropical environments of Africa and in the experimental hybrids for resistance to all the four diseases. The majority of the experimental hybrids were resistant to the four diseases indicating high levels of resistance to these foliar diseases in the hybrids. This high level of resistance in the single cross hybrids could be attributed to the mode of resistance identified for PLS, GLS, NLB and common rust. Resistance to these diseases was shown to be controlled mainly by additive gene action, although non-additive gene action was also important. Predominance of additive gene effects was also reflected by high heritability estimates for PLS and GLS. This implied, therefore, that it is possible to use susceptible parents crossed with resistant parents to produce resistant hybrids. However most of the commercial hybrid checks evaluated were susceptible especially to PLS. This suggested the need to improve the parents of these hybrids for PLS disease resistance or to develop new hybrids with high levels of resistance to PLS. Generation mean analysis (GMA) results also

confirmed through the R x S crosses for PLS and GLS that resistance in the inbred lines used was predominantly controlled by genes with additive effects.

Therefore disease resistance for PLS, GLS, NLB and common rust can be incorporated through methods such as backcross breeding, pedigree breeding or recurrent selection. Pedigree breeding would be effective to increase the disease resistance in maize germplasm, especially when the heritability is high. Despite being laborious and time consuming, it is precise and easily observed in progeny plants for simply inherited traits. Backcross breeding would also be an important approach; however, its major drawbacks include the fact that despite the resistance genes being transferred, the new variety is not necessarily superior to the recurrent parent. In addition if there are any undesirable genes that are closely linked, these may be transferred along with the resistance genes (linkage drag). The procedure can also be time consuming and costly as hybridization has to be done with each backcross. Recurrent selection, on the other hand, has been useful in improving various maize traits within populations or in a cross between populations. It involves "*selection, self-pollination and production of progenies from the desirable plants, evaluation of the progenies to identify superior ones, and intercrosses or recombination among selected progenies*" (Chahal and Gosal, 2002). Several studies have reported successfully using recurrent selection to increase NLB resistance (Ceballos et al., 1991; Campana and Pataky, 2005; Carson, 2006). The number of cycles tends to vary depending on the type of recurrent selection adopted. It is usually used in long term breeding plans as it is time consuming and laborious.

Based on the review by Wisser *et al*. (2006), the presence of clusters of disease quantitative loci (dQTL) for multiple diseases was identified. From these distinct dQTL distributions for the different diseases, it was evident that certain breeding schemes would more suitable for certain diseases (Wisser *et al*., 2006). It may therefore be possible to breed for multiple disease resistance especially if the breeding schemes are the same. In addition, resistance QTL associated with NLB, GLS, PLS have been mapped in maize (Pratt *et al*., 2003) and this presents molecular marker assisted selection (MAS) as a potential strategy to improve resistance to these diseases.

The study also revealed that the use of one parent with resistance would provide adequate resistance for PLS and GLS in single cross hybrids. Therefore the non-additive gene action that was associated with reduced disease levels may be exploited in developing single cross maize hybrids among these inbreds when one of the parents is resistant.

The study also identified hybrids which performed well across or in specific environments. Hybrids which were stable and high yielding were H21 (CZL00009 x A16), H14 (A1220-4 x A16), S63 (SeedCo hybrid check), N72 (MP72/N3) and H26 (CZL00001 x A16). A16 is a line derived from CML312 and it clearly showed that it performs well across a number of environments; therefore it can be used for wide adaptation. Hybrids H1 (CML445 x A1220-4), H44 (CZL00009 x CML443) and H18 (CZL00009 x CZL00001) were identified by AMMI and GGE biplot analyses as unstable but high yielding. These hybrids actually performed well in ZAM09, UG09 and BF09 and are therefore suitable for specific adaptation.

9.4 References

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