

## Gene action determining *Phaeosphaeria* leaf spot disease resistance in experimental maize hybrids

J. Derera<sup>1\*</sup>, P. Tongoona<sup>1</sup>, B.S. Vivek<sup>2</sup>, N. van Rij<sup>3</sup> and M.D. Laing<sup>1</sup>

<sup>1</sup>African Centre for Crop Improvement, University of KwaZulu-Natal, Private Bag X01, Scottsville, Pietermaritzburg, 3209 Republic of South Africa

<sup>2</sup>CIMMYT-Zimbabwe, P.O. Box MP163, Mt. Pleasant, Harare, Zimbabwe

<sup>3</sup>Crop Protection, Cedara, KwaZulu-Natal Department of Agriculture and Environmental Affairs, Private Bag X9059, Pietermaritzburg, 3200 Republic of South Africa

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*Phaeosphaeria* leaf spot (*Phaeosphaeria maydis* Henn.) has the potential to cause substantial yield losses in maize. Maize is grown by small-scale farmers without fungicides; hence there is need to breed for resistance in regionally adapted germplasm. Little information about the gene action determining *Phaeosphaeria* leaf spot disease (PLS) resistance in African maize germplasm is currently available. This study was therefore conducted to determine the gene action controlling resistance to PLS in African maize germplasm. Seventy-two experimental hybrids were generated in eight sets according to a North Carolina Design II mating scheme. Experimental and check hybrids were evaluated in an 8 x 8 simple lattice design during the 2003/4 season, and in an 8 x 10  $\alpha$ -lattice design, with two replications, during the 2004/5 seasons at the Cedara and Rattray Arnold Research Stations, in South Africa and Zimbabwe, respectively. There was significant variation among hybrids for resistance. General combining ability (GCA) due to both male and female inbred parents were highly significant ( $P < 0.01$ ), whereas specific combining ability effects were not significant for PLS scores, indicating that mainly additive gene action conditioned PLS resistance in experimental hybrids. Significant differences between male and female GCA variances, suggested the importance of cytoplasmic inheritance for PLS resistance. Resistance was highly heritable indicating that selection could be used to improve the resistance in this germplasm.

**Keywords:** Inheritance, maize, *Phaeosphaeria maydis*, resistance

\* To whom correspondence should be addressed (E-mail: Dereraj@ukzn.ac.za)

### Introduction

*Phaeosphaeria* leaf spot disease (PLS), caused by the fungus *Phaeosphaeria maydis* (Henn.), has the potential to cause substantial yield losses in maize (Pegoraro *et al.*, 2002; Carson, 2005b). Over the past six years, PLS incidence has been increasing in South Africa, Zimbabwe, Kenya and Cameroon. It is mainly prevalent in high rainfall, cool and high altitude areas (Carson, 2001; Silva & Moro, 2004). Although yield loss assessments have not been conducted in southern Africa, substantial yield losses have been reported in Brazil. Pegoraro *et al.* (2002) reported significant correlation ( $r = 0.45$ ) between grain yield reduction and PLS severity. Paccola-Meirelles *et al.* (2001) reported a yield reduction of 63% in Brazil, while Carson (2005b) reported yield losses of 11-13% in the USA as a result of PLS damage. This indicates that PLS has the potential to cause economic damage if susceptible hybrids are grown in southern Africa. Grain yield losses are caused by accelerated leaf senescence; reduced development period, and decreased grain size and weight in susceptible hybrids (Paccola-Meirelles *et al.*, 2001).

Success in breeding for resistance to PLS in maize would be accelerated if the mode of inheritance of resistance and gene action in regional germplasm were established. Carson (1999; 2001) reported that inbred lines of B73-type were more susceptible to PLS than those related to Mo17. These inbred lines represent the two major heterotic groups used in USA hybrid programmes. Additive gene action has been found to be predominant, while dominant gene action has been reported to play a very minor role in controlling PLS resistance in American and Brazilian germplasm (Carson,

2001; Silva & Moro, 2004). Carson (2001) reported that incomplete dominance was conferred by about four genes in Mo17; whereas Pegoraro *et al.* (2002) reported two major independent genes that controlled PLS resistance in an additive manner in Brazilian maize. This implies that single cross hybrids will be sufficiently resistant when both parents are resistant to PLS.

Few studies of inheritance of PLS resistance have been conducted, using American and Brazilian materials, which do not have a direct application to southern Africa. In southern Africa, the area grown to USA and Brazilian germplasm is not of any significance; hence the need to breed for resistance in regionally adapted germplasm. Management and environmental conditions in the Americas are also likely to be different from those in southern Africa. This study was therefore conducted to determine gene action controlling PLS resistance in experimental hybrids among southern African inbred lines.

### Material and methods

#### Germplasm

Parent inbred lines comprised a sample from eight major heterotic groups and their derivatives (Table 1) that are widely used in breeding programmes in southern Africa. Gevers & Whyte (1987) and Mickelson *et al.* (2001) present detailed descriptions of these heterotic groups. Inbred lines were divided into eight subgroups of three each; hence, three inbred lines in one subgroup were used as females and crossed with three inbred lines from another subgroup, used

**Table 1** Pedigrees and *Phaeosphaeria maydis* disease (PLS) resistance of parent inbred lines used in a design II mating scheme

Inbred	Heterotic group <sup>+</sup>	Pedigrees for the non-proprietary inbred lines (Proprietary lines are coded in brackets)	Resistance level <sup>†</sup>
CML312	A	S89500F2-2-2-1-1-B*5	MR
C24	A	[P501c2/[EV7992#/EV8449-SR]C1F2-334-1(OSU8i)-1-1-X-X-B-B]-4-1-1-1-2-1-B	MR
B16	I	[MSR123XI137TN 9-2-4-X-3/LZ956441]-B-1-5-5-B-4-B-B-B-B	MR
A13	A	[[EV7992]C1F2-430-3-3-X-7-B-B/CML202]-6-2-2-3-B-B	MS
A14	A	Z97SYNGLS(A)-F2-97-1-1-1-B	MS
CML442	A	[M37W/ZM607#bF37sr-2-3sr-6-2-X]-8-2-X-1-BBB	MS
CML489	AB	(CML202/LPSC3H297-2-1-1-2-2-#)-B-3-1-1-8-BB	MS
A26	I	(L55XL66)	MS
B11	K	(L10 X L12)	MS
B12	K	(L9XL13)	MS
B19	K	(L20XL88)	MS
B20	K	(L73XL68)	MS
B24	SC	(L22XL45)	MS
B17	B	[LZ956441/LZ966205]-B-3-4-4-B-5-B-B-B-B	R
CML395	B	90323(B)-1-B-1-B*4	R
CML444	B	P43C9-1-1-1-1-1-BBB	R
CML488	B	DTPWC8F31-4-2-1-5-BBB	R
B10	K	(L11XL15)	R
A7	M	(L67XL77)	R
A15	N3	[CML197/N3//CML206]-X-32-1-4-B-B-B-B	R
A9	NAW	(L89XL42)	R
B22	SC	(L21XL46)	R
B23	SC	(L78XL47)	R
CML445	AB	[[TUXPSEQ]C1F2/P49-SR]F2-45-7-5-1-BBB	S
B18	B	Z97SYNGLS(B)-F2-188-2-1-3-B	S
B21	K	(L70XL18)	S
A8	M	(L90XL33)	S

<sup>+</sup>Heterotic group A includes materials related to N3 (Salisbury white), NAW, Tuxpeno, Kitale and B73; heterotic group B consists of materials related to SC (Southern Cross), K (pride of saline), Eto, Ecuador and Mo17. <sup>†</sup>Susceptible lines had above mean PLS scores, while resistant lines had below mean scores; MS = Moderately susceptible, MR = moderately resistant; R = resistant and S = susceptible

as male parents, to form hybrids in nine sets, according to a North Carolina Design II mating scheme (Comstock & Robinson, 1948; 1952). Each inbred line was used once as a female parent in one set and once as a male parent in another set. Seventy-two hybrids were generated from eight sets (i.e., eight sets × nine hybrids each); but the ninth set failed to produce adequate seed for evaluation in trials.

### Experimental design

Hybrids were evaluated at Cedara (1076 m altitude) in South Africa, and at the Rattray Arnold Research Station (RARS; 1350 m altitude) in Zimbabwe. Owing to inadequate seed for some crosses, only 64 hybrids, comprising 57 experimental and seven check hybrids, were evaluated in an 8 × 8 simple lattice design, in the 2003/4 season. Eighty hybrids, comprising 72 experimental and eight hybrid checks, were evaluated in 8 × 10 α-lattice designs with two replications, in the 2004/5

season. The trial consisted of two-row plots with 32 plants at both sites, but plants were spaced at 50cm within rows and 80cm between rows at Cedara, and 25 cm within rows and 75 cm between rows at RARS. Populations of 44000 and 53000 plants ha<sup>-1</sup> were established at Cedara and RARS, respectively. Fertiliser was applied at the rate of 120 kg N, 33 kg P, 44 kg K at Cedara; and 208 kg N, 35 kg P, 21 kg K ha<sup>-1</sup> at RARS, respectively. Total rainfall amount was 853 mm in the 2003/4 and 885 mm in the 2004/5 season at Cedara. At RARS rainfall recorded was 711 mm in 2003/4 and 826 mm in the 2004/5 season. Standard cultural practices, including hand planting, hand weeding and application of herbicides were followed, but fields were left to natural disease infection. Disease development was monitored every fortnight, from tassel emergence. Disease severity was assessed at 50% silk emergence and at hard dough stages, based on visual assessment of the whole plot by estimating (i) percentage leaf area diseased

or necrotic (% LAD) and (ii) a rating scale of one to nine. In this scale, one = no disease or traces of it; three = lesions present on lower leaves but little or no disease above the ear leaf; five = disease present on most leaves with some lower leaves dead; seven = lower leaves dead and numerous lesions on all upper leaves, and nine = nearly all leaf tissue dead or necrotic (Munkvold *et al.*, 2001).

### Statistical analyses

General analyses of variance were performed for PLS scores of all hybrids including checks. Genetic analyses for PLS scores of experimental hybrids were then performed in SAS (SAS Institute, 1997) as a fixed effects model across two locations (Hallauer & Miranda, 1988) for each season as follows:  $Y_{ijkpq} = \mu + S_p + gi(S_p) + g_j(S_p) + h_{ij}(S_p) + E_q + r_k(SE)_{pq} + (ES)_{pq} + (Eg)_{iq}(S_p) + (Eg)_{jq}(S_p) + (Eh)_{ijq}(S_p) + e_{ijkpq}$ ; where  $i = 1, 2, 3$ ;  $j = 1, 2, 3$ ;  $k = 1, 2$ ;  $p = 1, 2, 3, 4, 5, 6, 7, 8$ ;  $q = 1, 2$ ; and  $Y_{ijkpq}$  denotes the PLS score of the hybrid of a mating of the  $i^{\text{th}}$  female line, the  $j^{\text{th}}$  male line, in the  $k^{\text{th}}$  block, within set  $p$  and in the  $q^{\text{th}}$  location. The terms of the model were defined as follows:  $\mu$  = Grand mean;  $S_p$  = the average effect of the  $p^{\text{th}}$  set;  $g_i(S_p)$  = GCA effect common to all hybrid of the  $i^{\text{th}}$  female line nested within  $p^{\text{th}}$  set;  $g_j(S_p)$  = GCA effect common to all hybrid of the  $j^{\text{th}}$  male line nested within  $p^{\text{th}}$  set;  $h_{ij}(S_p)$  = SCA effect specific to hybrid of the  $i^{\text{th}}$  female and  $j^{\text{th}}$  male nested within  $p^{\text{th}}$  set;  $E_q$  = average effect of  $q^{\text{th}}$  location;  $r_k(SE)_{pq}$  = effect of the  $k^{\text{th}}$  replication nested within the  $p^{\text{th}}$  set and  $q^{\text{th}}$  location;  $(ES)_{pq}$  = interaction between set effects and the location;  $(Eg)_{iq}(S_p)$  and  $(Eg)_{jq}(S_p)$  = interaction between location and GCA nested within sets;  $(Eh)_{ijq}(S_p)$  = interaction between location and SCA nested within sets;  $e_{ijkpq}$  = pooled error or random experimental error. Variance components were estimated using REML in GenStat (Lawes Agricultural Trust, 2006). Heritability was estimated using the formula  $h^2 = 2\sigma_m^2 / (\sigma^2_{re} + \sigma^2_{fme}/e + 2\sigma_{me}^2/e + \sigma_{mf}^2 + 2\sigma_m^2)$  because the parents were almost fully inbred (beyond  $S_8$  generation); where  $\sigma_m^2$  = GCA male variance;  $\sigma^2$  = random error variance;  $\sigma_{mf}^2$  = SCA variance;  $\sigma_{fme}^2$  = location  $\times$  SCA variance;  $\sigma_{me}^2$  = location  $\times$

GCA male variance;  $r$  = number of replications and  $e$  = number of locations. Additive ( $\sigma_A^2$ ) and dominance variances ( $\sigma_D^2$ ) for PLS scores were estimated using the formula:  $\sigma_m^2 = \sigma_f^2 = \text{Covariance of half sib families (HS)} = \frac{1}{2}\sigma_A^2$ , and  $\sigma_{mf}^2 = \text{covariance of full sib families} - \text{covariance HS}_m - \text{covariance HS}_f = \sigma_D^2$ , because  $F = 1$  (where  $F$  is the inbreeding coefficient,  $m$  = male and  $f$  = female parents) (Hallauer & Miranda, 1988).

## Results and discussion

### Disease development and hybrid resistance

Hybrid resistance to PLS was expressed by reduced disease severity, reduced number and size of lesions on the plant tissues. Laboratory tests were conducted on maize leaf samples collected from Rattray Arnold Research Station and Cedara as part of a larger, ongoing study, and *Phaeosphaeria maydis* was confirmed as the causal agent (data not presented). Detailed work which includes samples from other areas in southern Africa is still in progress and results will be published in another paper. In the field, the first lesions were observed on the leaves above the ear in some entries but in most hybrids on the lower leaves. This suggests that the fungus was mainly spreading from the soil to the lower leaves of the host, but that there was also an airborne spread. Disease had a highly uniform distribution within the trial, especially during the 2004/5 season, possibly because the crop was grown under zero tillage and following another maize crop. Disease development was highly affected by the environment (Table 2), indicating that disease incidence and severity may differ between locations and seasons, and between seasons within locations. Severity of PLS was higher during 2004/5 season than 2003/4 season at Cedara and RARS. Disease scores ranged from one to five during 2003/4; and from one to seven during 2004/5 seasons, at both locations, which was adequate to discriminate the hybrids for resistance. Percent leaf area diseased (%LAD) also ranged significantly between 0.3% for the most resistant hybrids, and 45-50% for the most susceptible hybrids ZS255, SC513 and B24/B18 (data not shown), which is comparable to findings in Brazil. Pegoraro

**Table 2** Analyses of variance for *Phaeosphaeria maydis* disease scores at Cedara and Rattray Arnold Research Stations during 2004/5 and 2003/4 seasons

Source of Variation	2004/5 Season			2003/4 Season		
	d.f.	MS	P	d.f.	MS	P
Location	1	110.967	**	1	63.549	**
Set	7	5.748	**	7	15.754	**
Location $\times$ Set	7	4.976	**	7	7.787	**
Replication/Set/Location	16	2.516	ns	16	0.598	ns
GCA Female/Set	16	5.514	**	15	2.525	**
GCA Male/Set	16	3.014	**	15	2.454	**
SCA /Set	32	0.493	ns	19	0.933	ns
Location $\times$ GCA Female/Set	16	1.793	**	15	1.465	ns
Location $\times$ GCA Male/Set	16	0.575	ns	15	1.587	ns
Location $\times$ SCA/Set	32	0.886	ns	19	0.914	ns
Pooled error	128	0.584		98	0.892	

\*\* Significant at  $P \leq 0.01$

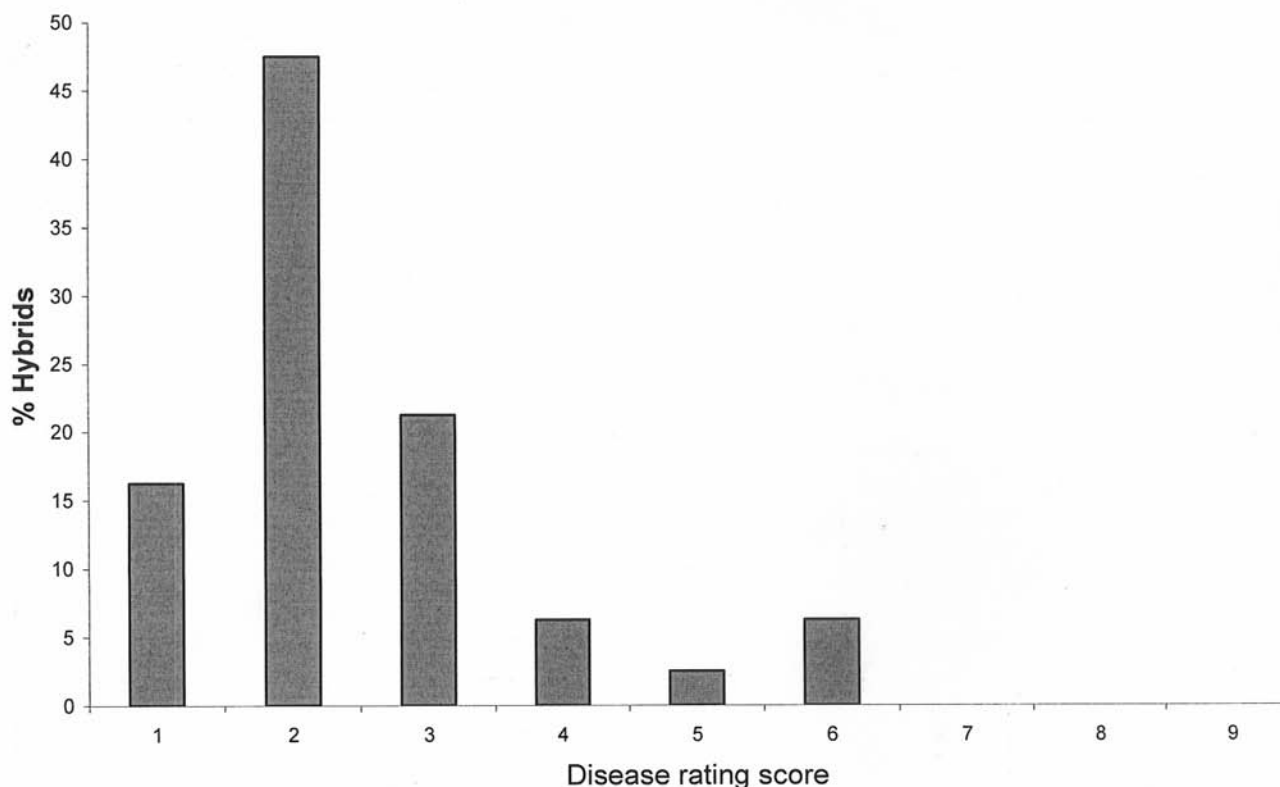
*et al.* (2002) reported 4.3% to 67% of leaf area diseased by PLS at 30 d after flowering in Brazil. In the current study, disease was first observed at the 50% silk emergence stage at both locations, which is consistent with literature from Brazil (da Silva & Moro, 2004). This late arrival in the crop cycle increases the chances that the disease severity could remain low and cause little yield loss (Carson, 2005b). Under favourable conditions, the onset and development of PLS may be relatively early (occurring at the vegetative stage), such that severe necrosis occurs, reducing photosynthetic area and grain filling (da Silva & Moro, 2004).

The frequency distribution of the PLS scores in the 80 maize hybrids was skewed towards resistance. Most hybrids (64%) had a PLS rating of  $\leq 2$  (Figure 1), indicating a high level of resistance; but only 16% of the 80 hybrids displayed trace symptoms of PLS. About 15% of the hybrids had a score of four to six and could be regarded as moderately susceptible to susceptible, while 21% had a moderate score of three for PLS across the locations. While there were no hybrids with scores of seven to nine across locations (Figure 1), some check hybrids (SC513 and SC403) had high disease levels, with rating scores of seven at both Cedara and RARS during the 2004/5 season (Table 3). Three standard hybrids (SC403, ZS255 and SC513) were among the most susceptible (Table 3), but two standard hybrids (R201 and R215) were ranked among the resistant, with PLS scores of two, although ranking did not put them among the top 10 hybrids (data not shown). The most resistant experimental hybrids (the top two hybrids) had one resistant parent (B23) in common, whilst the lines B22, B16, CML488 and CML444 were also constituents of the most resistant hybrids (Table 3), indicating that they can be used as sources of PLS resistance in breeding programmes. The most susceptible experimental hybrids (the

bottom 10 hybrids) had three common susceptible parents (B24, B18 and CML445) (Table 3), suggesting that these lines require improvement for PLS resistance. Although line GCA effects were confounded with sets, the lines B23, B22, CML488 and CML444 displayed significant contribution to resistance in their hybrids (Table 3).

### Gene action

There was a highly significant positive correlation between %LAD and the rating scores for PLS ( $r = 0.95$ ), indicating that any of the two could be used to rank the hybrids, therefore for the sake of brevity only the rating scores data are presented. Results indicated that hybrid resistance to PLS was controlled by additive gene action. Male GCA and female GCA effects were highly significant ( $P < 0.01$ ) for PLS scores (Table 2). Dominance variances were not estimated because the SCA variances were not significant for PLS scores (Table 2) during both the 2003/4 and 2004/5 seasons. These results were consistent with previous findings in tropical and temperate germplasm. The GCA variance was reported to be predominant over SCA in controlling PLS resistance in Brazil and the USA (Carson, 2001; Pegoraro *et al.*, 2002; Carson, 2005a). In east and southern Africa, Vivek *et al.* (2002) reported that GCA accounted for 65% of variation. Generally, the most resistant hybrids were crosses between resistant lines, while the most susceptible hybrids were crosses between susceptible lines (Table 3), which provide further evidence for the importance of additive gene action in controlling PLS resistance in these experimental hybrids. Only the environment  $\times$  female GCA interaction was significant ( $P < 0.01$ ) for PLS scores during the 2004/5 season, suggesting that selection should be conducted across sites. However, the environment  $\times$  GCA interaction was not significant during



**Figure 1** Frequency distribution of *Phaeosphaeria maydis* scores in 80 maize hybrids

**Table 3** *Phaeosphaeria maydis* disease (PLS) scores of the top10 and bottom 10 of the 80 maize hybrids at Cedara and Rattray Arnold during 2004/5 season

Hybrids	Hybrid Category	Location		Across Locations	
		Cedara (Score)	RARS (Score)	(Score)	Rank
<b>Top 10 hybrids</b>					
B23/B16	RXMR	1.0	1.1	1.1	1
K64R/B23	RXR	1.1	1.1	1.1	2
B22/B17	RXR	1.2	1.0	1.1	3
B20/CML444	MSXR	1.4	1.0	1.2	4
B12/B22	MSXR	1.3	1.1	1.2	5
B22/B16	RXMR	1.4	1.1	1.3	6
CML488/C24	RXMR	1.4	1.1	1.3	7
B16/CML312	MRXMR	1.7	1.0	1.3	8
CML444/A26	RXMS	1.6	1.1	1.4	9
B21/CML488	SXR	1.6	1.1	1.4	10
<b>Bottom 10 hybrids</b>					
B18/CML312	SXMR	4.6	3.0	3.8	71
B24/B16	MSXMR	5.6	2.4	4.0	72
K64R/B24	RXMS	4.5	4.0	4.3	73
CML445/A7	SXR	4.7	4.4	4.6	74
B11/B24	MSXMS	4.1	6.5	5.3	75
CML445/A8	SXS	5.1	5.9	5.5	76
SC513	Check hybrid	7.1	4.6	5.9	77
B24/B18	MSXS	6.3	5.5	5.9	78
SC403	Check hybrid	5.1	7.0	6.1	79
ZS255	Check hybrid	5.9	6.6	6.3	80
<b>Statistics</b>					
Mean		2.9	2.1	2.5	
SED		0.6	0.6	0.5	
LSD <sub>0.05</sub>		1.7	1.7	1.7	

R = resistant, S = susceptible and M = moderate

the 2003/4 season, which suggests minimum genotype  $\times$  environment interaction. Low genotype  $\times$  environment interaction effects were also reported in the USA (Carson, 2001; 2005b).

Clearly, female parents accounted for the greater part of the additive variance whereas the non-additive variance was not significant for PLS resistance. The estimates of GCA variances ( $\sigma^2_m$  and  $\sigma^2_f$ ) were 0.1812 ( $\pm 0.0815$ S.E.) and 0.2921 ( $\pm 0.1534$ s.e.), respectively, which are equivalent to the additive variances ( $\sigma^2_A$ ) of 0.3624 and 0.5842 for PLS scores, due to male and female parents, respectively, during the 2004/5 season. During 2003/4 season,  $\sigma^2_m$  and  $\sigma^2_f$  were 0.0664 ( $\pm 0.1036$ S.E.) and 0.1179 ( $\pm 0.1143$ S.E.), respectively, for PLS scores, which is equivalent to the additive variance of 0.1328 and 0.2358, due to male and female parents, respectively. With fixed effects models, Kang (1994) suggested the use of the GCA to SCA sum of squares to determine the relative importance of additive to non-additive gene action. In total, GCA accounted for 90%, with female GCA explaining 58% and male GCA 32% of cross sum of squares for PLS

scores in the 2004/5 season. During the 2003/4 season, GCA accounted for 76%, with female GCA contributing 40% and male GCA 36% of the variation. Predominance of female GCA over male GCA suggested the importance of cytoplasmic influence in the inheritance of PLS resistance in this regional germplasm. Breeders have to make a critical decision about which parent should be used as female when developing single cross hybrids. Previous studies in Brazil and the USA have not investigated or reported the role of maternal influence on the inheritance of PLS resistance in maize hybrids.

#### Heritability

Although heritability ( $h^2$ ) of PLS resistance was relatively high, results indicated that the time of disease rating is critical. Late disease ratings (at the hard dough stage) appeared to give higher  $h^2$  estimate than earlier disease ratings made just after flowering with reduced standard error. Estimated  $h^2$  was 52% ( $\pm 39\%$  S.E.) and 67% ( $\pm 15\%$  S.E.) for first and second rating scores, respectively. Heritability estimates for the first and second %LAD estimates were 62% ( $\pm 35\%$  S.E.) and 73% ( $\pm 19\%$  S.E.), respectively. High  $h^2$  estimates of 55-85% for PLS scores were previously reported (Carson, 2001; Vivek *et al.*, 2001; Carson, 2005a). High  $h^2$  estimates in this study were a reflection of the high GCA variance and suggested that resistance could be improved by selection.

#### Conclusions

Although the parent lines used in crosses were a representative sample of the major heterotic groups used in the regional breeding programmes, interpretations of results from this study was limited to this particular set of 27 inbred lines and their crosses; and some eight commercial check hybrids (parentage not known). General combining ability variance was highly significant, whereas SCA was not significant for PLS scores, indicating predominance of additive gene action in controlling PLS resistance in this set of experimental maize hybrids, which confirmed previous findings in Brazil and the USA. Significant differences between male and female GCA variances for PLS scores, suggested the importance of cytoplasmic inheritance for PLS resistance, which has not been previously reported or investigated. Reflective of the high additive variance, resistance was highly heritable which suggests that PLS resistance could be improved by selection in this set of regional maize germplasm.

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