

Full Length Research Paper

Genetic analysis of resistance to maize streak virus disease in dwarf maize germplasm

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Dwarf maize (*Zea mays* L.) hybrids grown in Zimbabwe have limited resistance to maize streak virus disease (MSD). Combining ability studies are important in revealing a genotype's utility in hybrid combinations. We investigated combining abilities and mode of inheritance of resistance to MSD in dwarf maize germplasm. A six-parent half diallel cross was conducted to generate fifteen F₁ hybrids, which were evaluated in a replicated field trial under an artificial epidemic of MSD. Scoring for MSD, development was done two weeks after inoculation and at flowering. Significant differences ($p < 0.05$) for resistance to MSD were observed among the F₁ progeny. These differences were attributed to both general combining ability (GCA) and specific combining ability (SCA), which were both significant ($p < 0.05$). GCA was more important than SCA as suggested by the large ratio (2.51) of GCA and SCA variance components. Additive gene effects were therefore predominant in the inheritance of resistance to MSD. Heritability of resistance to MSD was high (71%), suggesting that only a few major genes were involved. We believe that this is the first time that genetic analysis of resistance to MSD has been reported for dwarf maize germplasm.

Key words: Dwarf maize varieties, maize streak virus disease (MSD), general combining ability (GCA), specific combining ability (SCA), gene action.

INTRODUCTION

Maize (*Zea mays* L.) is the most important staple crop in Southern Africa, accounting for more than 50% of all the calories consumed in these countries (Byerlee and Eicher, 1997). In Zimbabwe, most of the maize is produced by smallholder farmers who have limited access to financial resources, the latest technologies, and land. Inadequate production of maize often results in food insecurity at household and national levels. The reasons for inadequate production are many, the most common being drought, poor soil fertility and, inadequate control of pests and diseases. Maize streak virus disease (MSD) is

one of the major diseases that can potentially cause a total crop failure on susceptible varieties during severe epidemics, and is reported to cover an estimated 60% of the total arable area in Africa (DeVries and Toenniessen, 2001). The disease is endemic throughout the maize producing areas in Africa. The economic impact of MSD to national economies in sub-Saharan Africa has been estimated to be between US\$120 million and US\$480 million per year (Martin and Shepherd, 2009). Use of resistant varieties is recognized as the most effective, economically viable and environmentally benign method of controlling this disease, especially for resource-poor farmers who cannot afford chemical control (Ngwira and Pixley, 2000; Lagat et al., 2008).

The African Centre for Fertilizer Development (ACFD) has a dwarf maize breeding programme on the outskirts of Harare, Zimbabwe. Three dwarf maize hybrids have

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thus far been released commercially from this programme (AC 71, AC 31 and AC 133). There are numerous dwarfing genes that have hitherto been identified, such as the dwarf mutants (dwarf1–dwarf9; Cassani et al., 2009) and the brachytic mutants (br1–br3; Cassani et al., 2010). Several of the dwarf mutations have not been used to improve yield because of abnormal phenotypes that they produce. Dwarf germplasm at ACFD utilize the brachytic-2 (br2) mutant that reduces plant height through shortening of the stalk internodes, without reducing cob size. While leaves of plants with this mutant become erect and darker (with a stay-green character), intermediate phenotypes were observed for plant height, ear height and leaf angle, when compared with tall phenotypes (Pilu et al., 2007). Pilu et al. (2007) suggested further exploration of this brachytic mutant when developing new maize hybrids. Mashingaidze and Chinhema (2004) showed that there were no significant differences in grain yield between six pairs of dwarf and tall near-isogenic, single-cross hybrids when they were evaluated in marginal and high-rainfall areas of Zimbabwe. Mwale (1999) reported that dwarf hybrids from Zimbabwe yielded more than some Zambian tall varieties in experiments carried out in Zambia.

Dwarf maize varieties could be important, especially for resource-poor communities farming in environments of marginal rainfall, and limited fertility. These varieties can be assumed to be more efficient in their use of water and nutrients as they do not produce unnecessary biomass. Mashingaidze and Chinhema (2004) reported that roots of dwarf hybrids were thinner and more fibrous than those of tall hybrids, and hypothesized that fine and fibrous rooting systems can be expected to be more efficient in extracting immobile nutrients and water because there will be more root surface area in contact with the soil. Dwarfing genes have caused plants to be, among other things, resistant to storm damage, lodging, and are therefore more tolerant to high populations when compared to normal-height varieties (Begna et al., 1999; Cassani et al., 2009). While hybrids developed by ACFD might have some of these comparative advantages, they have limited resistance to MSD. Concerted efforts are therefore needed to incorporate durable and stable resistance in these varieties.

Various reports about the inheritance of resistance to MSD in normal-height maize seem to indicate different modes of gene action. Resistance has been reported to be mainly controlled by either a single gene (Storey and Howland, 1967; Kytere et al., 1999), or additive gene action (Kim, et al., 1989; Nhlane and Caligari, 1997; Gichuru et al., 2011). The possible influence of modifier genes in changing types of gene action has been acknowledged (Pernet et al., 1999; Lagat et al., 2008). Gichuru et al. (2011) reported that GCA interacted highly with environments, and suggested that there is need for selecting specific parents for hybrid formation in specific agro-ecologies. There has not been any report on the

genetic analysis of resistance to MSD in dwarf germplasm. Such information can enable plant breeders to devise selection strategies for incorporation of dependable MSD resistance into inbred parents of dwarf hybrid varieties. We therefore sought to determine the combining abilities, and mode of inheritance of resistance to MSD in dwarf germplasm.

MATERIALS AND METHODS

F₁ progeny derived from six dwarf maize inbred parents were used in this study. Four of these inbred lines were susceptible to infection by the maize streak virus (MSV), and were code-named 21, 32, 51, and 72. The other two inbred lines were tolerant to (MSV), and were code-named ACL1 and ACL7. A six-parent half diallel cross was generated during the 2005/2006 summer season. The resultant fifteen F₁ hybrids were planted at the University of Zimbabwe farm (Thornpark Farm, 1 480 m above sea level, 31° East, 17° 31' South), about 13 km from Harare, during the 2006/2007 summer season. A randomized complete block design with three replications was used. Each plot consisted of five rows that were 5 m long. Inter-row spacing was 0.75 m, and in-row spacing was 0.3 m. The blocks were separated by a distance of 1.0 m. One row of a dwarf maize inbred line known to be susceptible to MSD (susceptible check) was planted 1.0 m from the plots. Five border rows were planted on each side of the trial to prevent the MSD from spreading outside the trial area. The plots received a basal compound fertilizer (7% N: 14% P₂O₅: 7% K₂O) at planting at a rate of 300 kg/ha and a topdressing of ammonium nitrate (34.5% N) at a rate of 250 kg/ha six weeks after crop emergence (WACE). Maize fields adjacent to the experimental plot were planted to tall maize varieties that were tolerant to MSD.

Plants were infested artificially with viriferous *C. mbiha* nymphs obtained from the CIMMYT-Zimbabwe Research Station. Infestation was carried out 10 days after crop emergence. Due to shortage of insects, every second plant in each row was infested, instead of the usual two insects per plant. Disease rating was conducted two weeks after infestation and also at flowering, that is, nine WACE. Disease rating was based on an individual whole plant basis using a modification of the disease scoring system used by the Regional Nursery Program (REGNUR) collaborators (Ngwira and Pixley, 2000). This means that each plant was rated after which the mean score for each plot was obtained. Data analysis was done using the mean score of each plot. Genotypes with mean scores of 1 to 3.4 were regarded as susceptible, those with scores of 3.5 to 6.4 were considered intermediate, while genotypes with scores 6.5 to 9 were resistant (Nhlane and Caligari, 1997).

MSD scores at flowering were subjected to analysis of variance. The observed variability for response to MSV infection was then partitioned into GCA and specific combining ability (SCA) effects. This was based on Griffing's Model 1 (GCA and SCA fixed), Method 4 (F₁ only) (Griffing, 1956). The formulas used to calculate the general and specific combining abilities were as described by Singh and Chaudhary (1985).

The basic model used in the analysis of variance was:

$$Y_{ijk} = \mu + \text{rep}_k + g_i + g_j + s_{ij} + e_{ijk}$$

Where Y_{ijk} = mean value of the k^{th} plot of the cross between the i^{th} and j^{th} parent; μ = overall mean; rep_k = replication or block effects g_i and g_j = GCA effects of the i^{th} and j^{th} parent respectively; s_{ij} = SCA effects of the cross between the i^{th} and j^{th} parent, and e_{ijk} = error peculiar to the ijk^{th} observation.

The narrow sense heritability was calculated as follows:

Table 1. Mean MSD scores (above the diagonal) and classification of response to infection by MSV (below the diagonal) for fifteen F₁ hybrids from a six-parent half diallel cross.

Line	21	32	51	72	ACL1	ACL7
21		1.0	1.0	1.8	7.0	6.7
32	S		2.0	1.7	5.2	5.0
51	S	S		1.0	5.2	4.8
72	S	S	S		6.2	5.0
ACL1	R	I	I	I		6.5
ACL7	R	I	I	I	R	

S, Susceptible (scores 1.0-3.4); I, intermediate (scores 3.5-6.4); R, resistant (scores 6.5-9.0).

Table 2. Mean squares and variance components for resistance to MSD based on diallel analysis according to Griffing's (1956) Model 1, Method 4.

Source	DF	MS	Expected MS
Replications	2	0.017 ^{ns}	
Crosses	14	15.774*	
GCA	5	12.64*	2.86
SCA	9	1.21*	1.14
Error	28	0.201	

*: Significant at $p < 0.05$, ns: Not significant at $p < 0.05$

$$h^2 = \frac{4 \sigma^2 \text{GCA}}{4 \sigma^2 \text{GCA} + 4 \sigma^2 \text{SCA} + \sigma^2 \text{error}}$$

The relative importance of additive and non-additive gene effects was determined using Baker's (1978) formula as follows:

$$\frac{2 \sigma^2 \text{GCA}}{2 \sigma^2 \text{GCA} + \sigma^2 \text{SCA}}$$

RESULTS

Even though some of the plants were not infested individually, disease pressure was satisfactory in all the plots with up to 80% of the plants in the plots showing symptoms of viral infection two weeks after infestation. The mean MSD ratings for F₁ hybrids at flowering time are presented in Table 1. The scores ranged from 1.0 for hybrids 32 × 21, 21 × 51 and 51 × 72 to 7.0 for hybrid 21 × ACL1. There were six susceptible hybrids (scores of 1 to 3.4), six hybrids that had intermediate resistance (scores of 3.5 to 6.4) and three resistant hybrids (scores of 6.5 to 9.0). The mean MSD scores for the three hybrid classes were 1.4, 5.2 and 6.7 for susceptible, intermediate and resistant F₁ hybrids, respectively. There was a significant difference ($p < 0.05$) between means of the three classes of response to infection by MSV. All hybrids generated from crosses between susceptible lines were susceptible. These were hybrids 21 × 72, 32 ×

21, 21 × 51, 51 × 72, 32 × 72 and 32 × 51. Resistant F₁ hybrids were generated from crosses between the two resistant lines, and also from crosses between the resistant lines and susceptible inbred parent 21. Intermediate resistance was shown by hybrids generated from crosses between resistant lines and susceptible lines, except line 21.

Analyses of variance showed significant differences ($p < 0.05$) among crosses for MSD scores (Table 2). These differences were attributed to both GCA and SCA effects, whose mean squares were also significant ($p < 0.05$). The GCA to SCA variance ratio was 2.51. Thus, the GCA variance was about two and half times larger than SCA variance, indicating that GCA effects were more important than SCA effects, though they were both significant. The relative importance of GCA and SCA, when calculated according to Baker (1978), gave a value of 0.83. This value was close to unity, giving further evidence of the importance of GCA when compared to SCA in determining the response of the F₁ progeny to infection by MSV. The narrow sense heritability estimate for resistance to MSD at flowering was 71%, which is relatively high. This high heritability estimate further supports the predominance of additive effects in the inheritance of resistance to MSD.

Estimates of GCA effects are presented in Table 3, along the diagonal. These were compared to determine the relative contribution of individual parents to MSD resistance as determined by MSD scores of their progeny. A negative GCA for a given parent indicated that crosses involving that parent were more susceptible than the average of F₁ crosses, thus it contributed towards greater susceptibility to MSD, whereas, a positive GCA indicated a contribution towards greater resistance. The most tolerant F₁ crosses had ACL1 as a parent, and this was because it had the highest positive GCA effect of 2.52. Considering the susceptible parents alone, inbred line 51 contributed the greatest susceptibility to its progeny, with a GCA effect of -1.51, while inbred line 21 contributed the least. Generally, resistant parents had positive GCA effects while susceptible parents had negative GCA effects. All the SCA effects were positive for these particular crosses. Crosses 21 × ACL1 and 21 × ACL7 had the highest SCAs of 5.08 and 5.13, respectively.

The mean grain yield for the crosses ranged from 0.21 t/ha for hybrid 32 × 51, to 9.25 t/ha for hybrid 72 × ACL7. Significant variation ($p < 0.05$) was found among the fifteen hybrids for grain yield. The mean grain yields of the three hybrid classes were 8.7, 7.66 and 0.69 t/ha for resistant, intermediate and susceptible classes of varieties, respectively. There were no significant differences ($p > 0.05$) between the mean grain yields of resistant hybrids and those with intermediate resistance, when each class was considered alone. However, the mean yields of these two classes (resistant and intermediate resistance) were significantly different from that of susceptible hybrids.

Table 3. Estimates of general combining ability (GCA) (diagonal) for the six parents, specific combining ability (SCA) (below diagonal) and yield (t/ha) (above diagonal) for 15 hybrids from a six parent half diallel cross.

Line	21	32	51	72	ACL1	ACL7
21	-0.63	0.56	0.51	1.56	7.01	8.34
32	1.61	-1.28	0.21	0.95	7.07	7.70
51	1.76	3.19	-1.51	0.38	9.01	5.79
72	2.28	2.61	2.06	-1.09	7.12	9.25
ACL1	5.08	4.06	3.86	4.57	2.52	9.00
ACL7	5.13	3.86	3.81	3.73	2.83	1.99

DISCUSSION AND CONCLUSION

Six F_1 hybrids were susceptible; six were of intermediate resistance, while three were resistant to MSD. The significance of both GCA and SCA effects indicated that both additive and non-additive effects were important. A preponderance of GCA effects suggested that additive gene effects were more important in the inheritance of resistance to MSD in the materials evaluated. This additivity of gene effects, in addition to a high narrow sense heritability of 71%, suggested that it should be possible to record genetic gains from selection for resistance to MSD from segregating generations since the trait can be transmitted to subsequent generations. In this case, it is important to carefully select parents with resistance to MSD, since their phenotypic performance provides a good indicator of their potential genetic contribution to their progeny (Falconer, 1989). Approaches to improvement that focuses on additive effects; such as recurrent selection and backcross breeding, can therefore be used to generate dwarf maize inbreds with resistance to MSD. The high heritability value further suggested that selection for MSV resistance can effectively be based on the visual scoring system that was used in this study.

Since additive gene effects were observed to be more important than SCA effects, it suggested that resistance was controlled quantitatively. However, the high heritability (h^2) estimate suggested that, though inheritance was quantitative, only a few major genes were involved, as opposed to numerous genes with minor effects. The quantitative nature of the inheritance of resistance to MSD means that resistance is likely to be effective, stable, and/or durable. Stability of the resistance to MSD in some resistant normal-height maize varieties was also reported by Mawere et al. (2006), who evaluated the resistance of resistant maize inbred lines to twenty MSV isolates. On the other hand, the fact that SCA effects were significant, even though they were less important than GCA effects, means that variability for resistance to MSD cannot be explained on the additive model alone. Non-additive gene effects, characterized by either intra-locus (dominance) or inter-locus (epistasis) interaction of alleles also contributed to the observed

variability, though to a lesser extent. These results reflect reports by Nhlane and Caligari (1997), who also observed significant GCA and SCA effects among F_1 offspring, as well as high narrow sense heritability in normal-height maize germplasm. The observed significance of non-additive gene action may imply that one could make genetic improvements through selection, followed by inbreeding and cross breeding. This way, hybrid combinations with superior resistance to MSD can be identified.

A comparison of the GCA effects of the six parents showed that line ACL1 had the highest (2.52) GCA effect. As a result, the mean performance of crosses involving ACL1 were the most resistant when compared to the mean performance of the crosses involving other inbred lines. In other words, line ACL1 transmitted the greatest MSD resistance to its progeny when compared to the other inbred lines. Inbred line ACL1 can therefore be used as a parent where resistance to MSD is required, for example in backcrossing schemes to convert susceptible lines to those with resistance to MSD. The SCAs of all the crosses were positive for resistance to MSD. This was probably because crossing brought together favourable alleles for resistance to MSD, coupled with non-additive effects. Hybrids 21 × ACL1 and 21 × ACL7 had the highest SCAs, indicating that these experimental hybrids were superior crosses in terms of resistance to infection by MSV.

Maize streak virus disease had a severe impact on yielding ability of some of the single cross dwarf maize hybrids. Zero yields were recorded in some plots that had susceptible hybrids. However, the effect of MSD could have been confounded by the different combining abilities of the inbred parents for yield. Hybrids with intermediate resistance to MSD were tolerant to the disease because, despite showing symptoms of viral infection, their yields were negligibly affected, and were not significantly different ($p > 0.05$) from those of resistant hybrids. Kyetere et al. (1999) and Gichuru et al. (2011) also described such genotypes with relatively high yields and intermediate levels of resistance as being tolerant to MSD. Even though line ACL1 was recommended for use where a parent with resistance to MSD is required, crosses involving line ACL7 out-yielded those involving line ACL1, except for the crosses involving the resistant lines with line 51. The hybrid ACL1 × 51 yielded more than ACL7 × 51. This suggested that while line ACL1 had a higher combining ability for resistance to MSD, line ACL7 might have a better combining ability for yield. Therefore, choice of suitable donor-parents for resistance to MSD should also consider other agronomic traits. Use of mating design models involving parents is recommended for future investigations in order to derive more accurate conclusions on the parents.

We conclude that, though both GCA and SCA effects were involved in the determination of resistance to MSD in the dwarf germplasm that was tested, a preponderance

of GCA effects showed that additive gene action was more important. Hybrid performance can therefore be predicted mostly on the basis of GCA, though SCA can be considered to improve precision of identifying hybrid combinations with superior resistance to MSD. Inbred lines ACL1 and ACL7 were identified as good combiners for resistance to MSD, and can be used as donor-parents in dwarf maize breeding programmes. Though resistance to MSD was shown to be inherited quantitatively, the high narrow sense heritability suggested that only a few genes were involved. Typical of quantitative resistance, the MSD resistance operating in these inbred lines is likely to be stable, durable, and unlikely to be overcome by new physiological races of MSV. The types of gene action and inheritance patterns observed were similar to findings from some normal-height maize germplasm. Uses of recurrent selection or backcrossing schemes that increase the frequency of favourable alleles are postulated to be effective when breeding dwarf maize for resistance to MSD.

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