

Project number	M191/11
Project title	Genetic characterisation of <i>Stenocarpella maydis</i> ear rot resistance in maize
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### **FINAL ABSTRACT**

*Stenocarpella* ear rot (diplodia) is of great economic importance in South Africa. It causes yield losses, downgrades grain and causes diplodiosis. It occurs sporadically especially under monoculture and/or conservation agriculture. Development of resistant cultivars is the most cost-effective control. The objective of the project was to identify QTL's for resistance to *Stenocarpella* ear rot. Three resistant maize inbred lines, namely CML 395, B37 and E739, were crossed with one susceptible inbred line MO17 to develop three mapping populations. The resultant F<sub>5</sub> recombinant inbred lines and their original parents plus two checks (PAN 53, resistant check and PAN6479, susceptible check) were planted at Bethlehem, Cedara and Greytown during the 2011/12 summer season. An (0,1) alpha lattice design with four replications was used. All plants were inoculated by applying 5 ml of powdered infested kernels in the leaf whorls two times prior to tasseling. Leaf samples were collected from representative plants of the parental lines and individual F<sub>5</sub> plants. Trials were manually harvested and disease score data collected from all the ears. There were significant ( $p < 0.05$ ) differences among entries for diplodia infestation at Cedara and Greytown but not at Bethlehem. Leaf samples from Cedara and Greytown will be used for QTL identification.

### **Keywords**

*Stenocarpella maydis*, Diplodia, ear rot, biotic stress, grain, yield, disease, fungus, sporadic

## INTRODUCTION

In South Africa, maize production is limited by both biotic and abiotic stresses (Van Rensburg *et al.*, 1997). Biotic stresses include pests, diseases and parasitic weeds. Abiotic stresses are mostly drought and low soil fertility.

*Stenocarpella* ear rot is a maize disease that is common in South Africa. It is also known as diplodia ear rot. It is a soil-borne and seed-transmitted disease (Smith and White, 1988) caused by *Stenocarpella maydis* (Berck). The fungus also causes stalk rot (Rheeder *et al.*, 1990; Flett, 1999). *Stenocarpella* ear rot can cause yield losses of up to 85% in years conducive to the outbreaks of the disease (Flett, 1999). In the 1980's there was an outbreak of ear rot disease in South Africa resulting in annual losses of about R400 million (Russouw *et al.*, 2002).

*Stenocarpella* ear rot occurs sporadically in the major maize production areas (Flett, 1990), particularly where farmers practice monoculture and/or conservation agriculture. This makes it difficult for farmers to prepare for its occurrence and control. Development of resistant cultivars is the most cost-effective control. Resistance to *Stenocarpella* ear rot is polygenic and difficult to select for under field conditions. The identification of molecular markers linked to QTL's would aid breeding efforts. By selecting directly at DNA level, breeding for a quantitative trait can be independent of environmental effects, making backcrossing possible. Rates of genetic gain twice as high as those of conventional breeding were reported for marker-assisted selection in maize. With marker-assisted selection it is possible to recover more than 95% of the recurrent parent's genome in three or less backcross generations. Marker-assisted backcrossing has huge benefits for maize breeding and is used for (i) selection of disease resistance genes or QTL's, (ii) elimination of unwanted regions of the donor-parent genome linked to the gene of interest and (iii) selection of unlinked regions of the recurrent-parent genome.

The long-term objective of this project was to develop and release resistant inbred lines to the maize seed industry. The short-term objective was to identify QTL's for resistance to *Stenocarpella* ear rot.

## **MATERIALS AND METHODS**

Three resistant maize inbred lines, namely CML 395, B37 and E739, were crossed with one susceptible inbred line MO17 to develop three mapping populations. For each population, the single-seed descent method was used to advance the  $F_1$  progeny to the  $F_5$  generation using a summer nursery at Cedara and a winter nursery at Makhathini research station. The resultant  $F_5$  recombinant inbred lines and their original parents plus two checks (PAN 53, resistant check and PAN6479, susceptible check) were planted at Bethlehem, Cedara and Greytown during the 2011/12 summer season. An (0,1) alpha lattice design with four replications was used. The inoculum for artificial infestation of individual plants was prepared in accordance with techniques recommended by ARC-GCI pathologists, using an aggressive isolate from Viljoenskroon. All plants were inoculated by applying 5 ml of ground-infested kernels in the leaf whorls two times prior to tasseling. Basal fertiliser, 3:2:1 (25%) + Zn, was broadcasted prior to planting at the rate of 300 kg/ha. Top dressing fertiliser, LAN (28%), was banded at the V6 stage at 300 kg/ha. Kombat granules were applied into the leaf whorls for stalk borer control. A fungicide was sprayed to control gray leaf spot disease. Pre-emergence and post-emergence herbicides plus manual weeding were used for weed control. Leaf samples were collected from representative plants of the parental lines and individual  $F_5$  plants following biotechnology laboratory protocols.

Data were collected on days to 50% anthesis, days to 50% silking, plant and ear height, husk cover (closed or open tips), ear aspect (drooping or upright), prolificacy and stem lodging. At all locations trials were manually harvested and disease score data collected from all the ears. Field weight, grain weight and grain moisture content were also recorded. Data were subjected to analysis of variance per location using Genstat. When found significant per location combined ANOVA was performed (Gomez and Gomez, 1984). Means were compared using least significant difference (LSD) at  $p = 0.05$ .

## RESULTS AND DISCUSSION

Effective ear rot infection takes place within the first three weeks after silking (Koehler, 1959). After three weeks ears become more resistant to the pathogen as they approach maturity. In this study the rainy season coincided with the silking period.

Diplodia infestation was not significantly different ( $P>0.05$ ) among entries at Bethlehem (Fig 1). The infestation levels were very low due to non-conducive weather conditions that did not favour growth of the fungus.

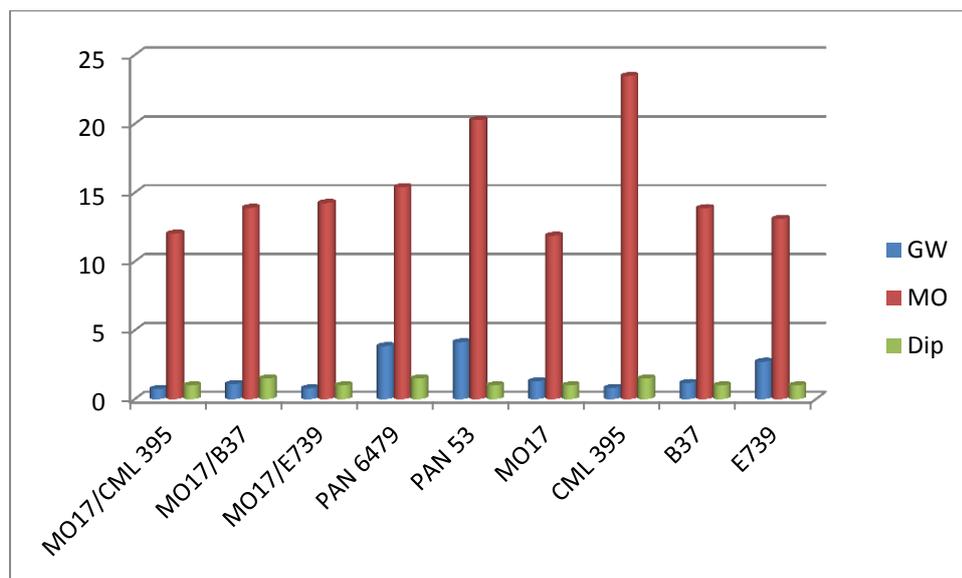


Figure 1: Moisture and diplodia infestation (%) at Bethlehem

Diplodia infestation was significantly different ( $P<0.05$ ) among entries at Cedara (Fig 2). This result was as expected because Cedara is a hot spot for ear rots due to its conducive weather conditions during summer. Dry conditions early in the season and warm ( $28 - 30^{\circ}\text{C}$ ), wet weather during the two to three weeks after silking favour ear infection of *S. maydis* (CIMMYT, 2004). CML395 had the highest infestation followed by PAN6479 (a susceptible check) and MO17/E739. The result for CML395 was unexpected because it had been described as resistant to ear rot.

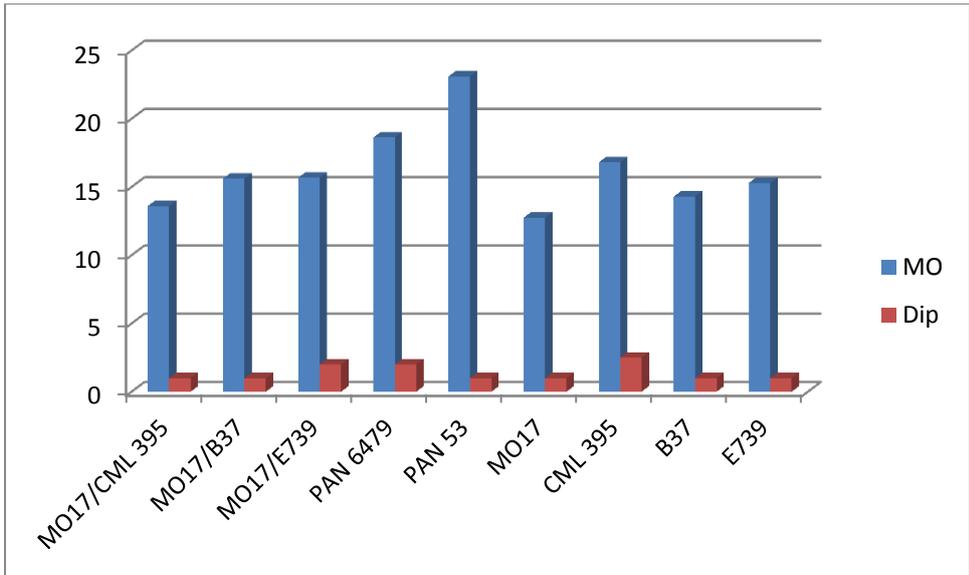


Figure 2: Moisture and Diplodia infestation (%) at Cedara

At Greytown there were significant ( $P < 0.05$ ) differences among entries for diplodia infestation. MO17 had the highest diplodia infestation followed by B37 and a cross between susceptible and resistant MO17/B37 (Figure 3). PAN 53 proved to be resistant as expected.

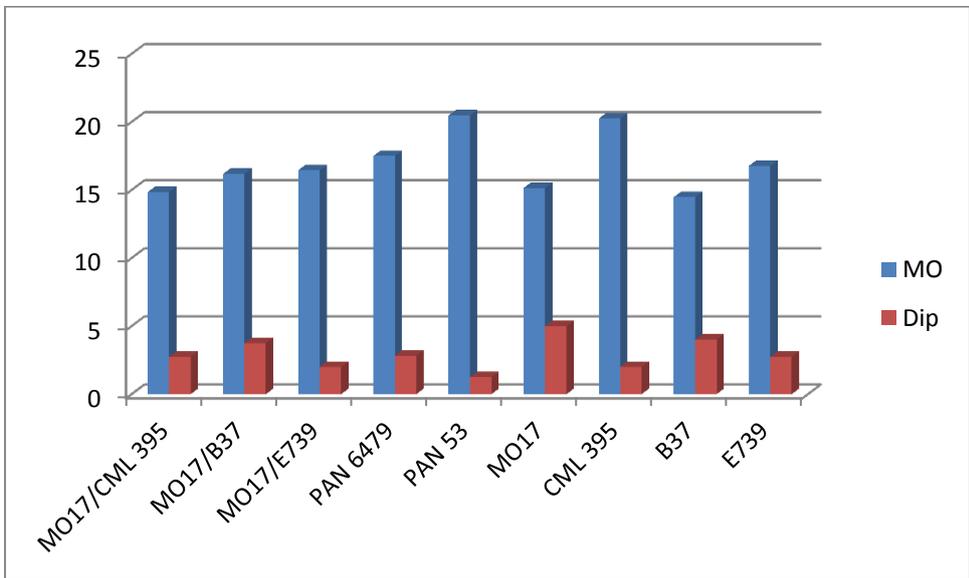


Figure 3: Moisture and Diplodia ear rot infestation (%) at Greytown

When analysed across locations, diplodia ear rot was more prevalent at Cedara followed by Greytown (Fig 4). There is evidence that *Stenocarpella maydis* ear rot resistance fluctuates from location to location in a given season and there is unlikely to be total resistance to

*Stenocarpella* ear rot. The infestation levels of the resistant check PAN 53 were the lowest at all locations. This shows that the data are quite reliable. Leaf samples from Cedara and Greytown will be used for identifying QTL's for resistance to *Stenocarpella* ear rot. This study confirmed a report by Rossouw *et al.*, (2002) that *Stenocarpella* ear rot is of polygenic inheritance, as different infestation ratings were observed from the three locations.

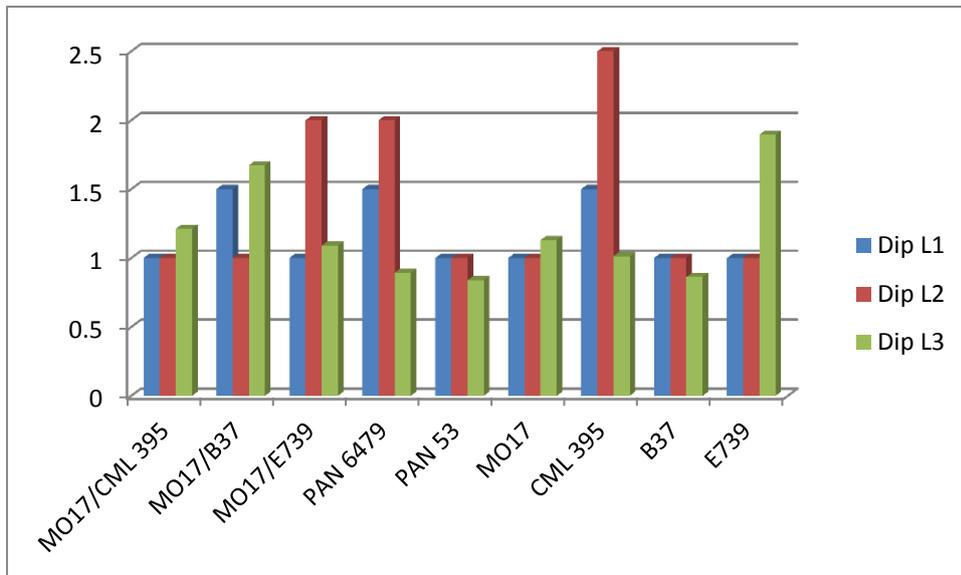


Figure 4. Diplodia infestation (%) across three locations

## **PUBLICATIONS**

MOREMOHOLO, L., 2009. Combining ability of maize inbreds for *Stenocarpella maydis* ear rot and grain yield: Oral presentation at Combined congress, Cape Town, South Africa.

MOREMOHOLO, L., 2009. *Stenocarpella maydis* ear rot resistance and yield performance among maize inbred lines and top-cross hybrids. Poster presentation at South African Association of Plant Pathologists, Cape Town, South Africa.

MOREMOHOLO, L., 2009. Diplodia (*Stenocarpella maydis*) ear rot. Referral poster for farmers and students. Pietermaritzburg, South Africa.

MOREMOHOLO, L., 2009. Stability analysis for grain yield and *Stenocarpella maydis* ear rot resistance in maize. Oral presentation at African Crop Science Society congress, Cape Town, South Africa.

MOREMOHOLO, L., 2010. Yield response and *Stenocarpella* ear rot reaction among selected maize inbred lines and top-cross hybrids. *Euphytica*, 174: 231 - 238.

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