FINAL REPORT 2017/18

DETAILS

Project number		P05000056 (100406)
Project title		Screening of South African maize hybrids (long and short season hybrids) for resistance to Fusarium ear rot and Gibberella ear rot and their resultant mycotoxins
Project manager		E Ncube
Co-worker(s)	Internal	BC Flett, TM Ramusi, DB Biya, F Mashinini, TJ Bass, NY Maila, MM Mahlobo, M Motlhatlego, KA Tantasi, MM Santhu
	External	Stellenbosch University, University of the Free State
Project status		Complete
Duration		01/04/2015 to 31/03/2018
Funder(s)		ARC/Maize Trust (Mycotoxin)

Final abstract

The aim of the project was to screen South African maize hybrids for resistance to Fusarium ear rot (FER), Gibberella ear rot (GER) and their associated mycotoxins. Long term growing and short growing season cultivars were screened for resistance to FER and GER in two separate trials during the 2015/16 and 2016/17 seasons. Potchefstroom trials were planted under dry land conditions with a high disease potential for *F. verticillioides* infection and FER, and a medium potential for *F. boothii* infection and GER. Vaalharts trials were under irrigation at a medium potential for FER development and a high potential for GER. In Cedara, trials were also under dry land conditions with medium potential for both GER and FER. The FER trials were inoculated with four high fumonisin-producing *F. verticillioides* isolates (MRC826, GCI340, GCI51 and GCI1608) while GER trials were inoculated with a mixture of four isolates of *F. boothii* (2881, M0100, M0002 and M0010). Maize ears from each FER and GER trial were rated for their respective disease symptoms after hand harvesting. Additive Main Effect and Multiplicative Interaction (AMMI) analysis was performed to determine hybrids that are resistant or susceptible to FER and/or GER using GenStat® 18.1. Results from the AMMI analysis for 2016/17 showed that FER incidence was significantly ($P < 0.05$) affected by G x E only on the FER *F. verticillioides*-uninoculated trial of the 59 long growing season genotypes. PAN4A-111 was the only hybrid which was highly susceptible in more than one locality. P2137B and LS8541BR were the most susceptible in Cedara and Potchefstroom, respectively, though the level of FER occurrence did not exceed 2.5%. Therefore, it is important that multi-seasonal studies are conducted to identify genotypes that can be recommended for planting in different climatic conditions since hybrid x season and season x location interactions are significant sources of variation for ear rot occurrence.

Keywords: Maize, Fusarium ear rot (FER), Gibberella ear rot (GER), Genotype x Environment interactions.

INTRODUCTION

Fusarium ear rot (FER) and Gibberella ear rot (GER) are major ear rot diseases associated with maize worldwide (Desjardins, 2006; Ncube *et al.*, 2011). The mycotoxins associated with FER are the fumonisins that are produced by *Fusarium verticillioides* and *F. proliferatum* in maize. In South Africa, GER is caused, exclusively, by *F. boothii*, a member of the *Fusarium graminearum* species complex (FGSC). Mycotoxins that are associated with GER are zearalenone and the type B trichothecenes [deoxynivalenol (DON) and its acetylated forms 3-acetly-deoxnivalenol (3-ADON) and 15-acetyl-deoxynivalenol (15-ADON); nivalenol (NIV) and its acetylated form 4-acetylnivalenol or fusarenone X (FX)]. Cultivar screening is a potentially important management intervention for the control of mycotoxin contamination of maize grain. Trials were planted in three localities namely, Potchefstroom, Vaalharts, and Cedara to accommodate different disease potentials and geographic spread rather than having too many localities that would have required more funding. Separate FER and GER trials were planted. FER trials were artificially inoculated with four high fumonisin-producing *Fusarium verticillioides* isolates mixed in equal proportions. GER trials were artificially inoculated with a mixture of four isolates of *F. boothii*. Maize ears from each FER and GER trial were rated for their respective disease symptoms upon harvest.

MATERIALS AND METHODS

Maize hybrids and planting of trials

Trials were planted in three localities namely: Potchefstroom, Vaalharts, and Cedara to accommodate different disease potentials and geographic spread. Long term growing season cultivars and short growing season cultivars (Appendix A) were screened in 2015/16 and 2016/17 season for resistance to FER and GER in two separate trials. These cultivars were the most commonly planted and available hybrids produced and marketed by different local seed companies. The maize cultivars were supplied by seed companies and made up into packages for experimental purposes at the ARC-GCI. Long and short season trials were planted separately to coordinate flowering dates and inoculations. The short season hybrids were planted 10-14 days earlier than the long season hybrids. There was a separate trial to screen for FER and for GER since these are two separate studies, therefore at each locality there were four trials that were planted per season [two Long term hybrids (FER and GER trials) and two Short season hybrids (FER and GER trials)]. The trials were repeated over two seasons (2015/16 and 2016/17 growing seasons). The trials were planted in a randomised complete block design and treatments replicated three times. Non-inoculated rows were planted next to the inoculated rows, and each row was 10 metres long. The trials were planted as provided in the plan for cultivar evaluations by the Cultivar Evaluation Program of the ARC-GCI. Soil samples were collected and analysed at the ARC-Institute of Industrial Crops Soil Analysis Laboratory in Rustenburg, South Africa to determine the quantity of fertiliser that needed to be applied in each season. Pre-emergence (S-metolachlor) and post-emergence (thiadiazine) herbicides were applied for weed control according to the manufacturer's instructions.

FER and GER inoculum preparation and inoculation

FER trials were artificially inoculated with four high fumonisin-producing *F. verticillioides* isolates (MRC826, GCI340, GCI51 and GCI1608, the last three are maintained at the ARC-GC culture bank). These isolates were then mixed in equal proportions to make up the inoculum. This cuts out variation due to possible selective reactions to a single isolate at specific localities. GER trials were artificially inoculated with a mixture of four isolates of *F. boothii* (2881, M0100, M0002 and M0010), these were provided by the Plant Pathology Department at Stellenbosch University. Pathogen selection was based on the locality where it was isolated and high level of mycotoxin production. The FER and GER inoculants were prepared, each isolate separately, in sterile Armstrong *Fusarium* medium (Booth, 1971). The concentration of the spore suspension was determined under a microscope using a Fuchs Rosenthal haemocytometer and diluted into the final 2×10^6 spores mL^{-1}. Tween 20 surfactant (polyoxyethylene 20-sorbitan monolaurate) was added to the spore suspension at a rate of 30 µL per litre to minimise clumping of spores. Isolates were mixed in equal proportion to develop the inoculum suspensions for both FER and GER. Maize ears were inoculated with FER and GER

pathogens by injecting 2 mL of each conidial spore suspension into the silk channel of each primary ear at the blister (R2) growth stage, as described by Small *et al*. (2012).

FER and GER ratings

FER and GER symptoms were visually rated on each ear and expressed as a percentage upon harvest (Ennerson and Hunter, 1980).

Statistical analysis

Additive Main Effect and Multiplicative Interaction (AMMI) analysis was performed to identify hybrids that are resistant or susceptible to FER and/or GER using GenStat® 18.1 (VSN, International, Hemel Hempstead, UK). The AMMI is a unified approach that fits the additive main effects of genotypes and environments by the usual analysis of variance and describes the non-additive parts by principal analysis fitted to the AMMI model. The AMMI is particularly effective in indicating adaptive responses, it displays genotype (G) main effects, environment (E) main effects, and genotype x environment (GE) interaction effects (Van der Merwe *et al*., 2013).

RESULTS

Additive main effects and multiplicative interaction (AMMI) analysis

The AMMI ANOVA for Fusarium ear rot (Table 1) of the FER *F. verticillioides*-uninoculated trial of 59 long growing season genotypes was tested in three environments, namely Cedara, Potchefstroom and Vaalharts in 2016/17. The analysis indicated that FER incidence was significantly ($P < 0.05$) affected by Genotype x Environment (Table 1). However, there was no significant ($P > 0.05$) G x E interaction for FER (inoculated) long growing season hybrids, GER (inoculated) long growing season hybrids, GER (uninoculated) long growing season hybrids, FER (inoculated) short growing season hybrids, FER (uninoculated) short growing season hybrids, GER (inoculated) short growing season hybrids, GER (uninoculated) short growing season hybrids. Data for the 2015/16 season could not be obtained due to a severe rodent infestation and damage at storage.

AMMI biplot-1 analysis

The AMMI biplot-1 analysis showed an association between the first interaction principal component axis (IPCA 1) and the means of genotype x environment (Fig. 1). Genotypes located in quadrant B and D of the biplot-1 had average FER that was higher than the grand mean of 0.38% FER whereas genotypes in quadrant A and C had average FER that were lower than the grand mean. Genotypes or environments with large IPCA 1 scores (either positive or negative) had high interactions while those with scores close to zero had low interactions and are considered stable. The genotypes falling close to the point of origin of the multiplicative axis (IPCA 1) had lower interaction with most of the environments and were therefore stable. Genotypes located beyond -1 and +1 showed a high interactive behaviour with the environments close to them and were unstable. These included G56 (P1659W), G58 (PAN5R-785BR) and G12 (P2137B).

Environments with IPCA 1 scores close to zero such as Vaalharts had little interaction with genotypes and had low discrimination of genotypes whereas Potchefstroom and Cedara with IPCA 1 scores beyond ±1 discriminated genotypes more effectively.

AMMI biplot-2 analysis

The AMMI biplot-2 analysis was plotted using IPCA 1 and IPCA 2 scores for genotypes and environments (Fig. 2). The AMMI biplot-2 indicated which cultivar was suitable in which environment. The AMMI biplot-2 showed that G12 (P2137B), G43 (LS8541BR) and G28 (P1745R) were furthest from origin and expressed a highly interactive behaviour (positive or negative) with specific environments.

The top five cultivars that were highly susceptible to FER in Cedara were G12 (P2137B), G56 (P1659W), G59 (P2880WYR), G50 (US9777) and G32 (IMP53-49B) whereas G43 (LS8541BR), G39 (DKC78-79BR), G48 (DKC76-61B), G36 (DKC78-45BRGEN), G49 (PAN4A-111) were highly susceptible to FER in Potchefstroom. G28 (P1745R), G57 (DKC77-77BR), G49 (PAN4A-111), G26 (BG3292) and G14 (PAN5A-182) were highly susceptible in Vaalharts. However, most cultivars were less susceptible to FER and the incidence of FER did not exceed the grand mean 0.38%.

Mycotoxin analysis and the quantification of mycotoxin-producing fungal species could not be completed on time due to more time taken milling 2880 samples. Milling has however been completed.

DISCUSSION

The significant Genotype x Environment interaction for FER in the FER *F. verticillioides*-uninoculated long season hybrids indicated that hybrids showed specific and wide adaptation to environments. PAN4A-111 was the only hybrid that was found to be highly susceptible in two localities namely Potchefstroom and Vaalharts. P2137B and LS8541BR were the most susceptible hybrids, with P2137B highly susceptible in Cedara while LS8541BR was highly susceptible in Potchefstroom though the level of FER occurrence did not exceed 2.5%. Hybrids that are resistant to FER (those with FER incidence of less than 0.38%) could form the core around which other Integrated Disease Management strategies, such as breeding for resistance, can be centred in order to reduce both Fusarium ear rot and Gibberella ear rot. Vaalharts was the most stable of the three environments indicating that it can be used to discriminate between stable and unstable hybrids. This is possibly due to high moisture availability in Vaalharts from irrigation. It has been reported that hybrids grown outside their range of adaptation are more susceptible to *F. verticillioides* infection and concomitant fumonisin production (Shelby *et al*., 1994; Miller, 2001). However, hybrid x season and season x location interactions are significant sources of variation for FER (Venturini *et al*., 2015). It is therefore important that multi-seasonal studies be conducted to identify genotypes that can be recommended for planting in different climatic conditions (Chimonyo *et al*., 2014). Elite maize cultivars should be adapted to specific as well as broad environments in order to have sustained disease resistance. The occurrence of disease in plants is a function of the host genotype, environment and pathogen; therefore, the study of G x E interactions is essential in identifying the suitability of maize cultivars in diverse environments.

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TABLES

Table 1 Additive main effects and multiplicative interaction ANOVA for FER on 59 long growing season genotypes planted in 2016/17.

Source	d.f.	s.s.	m.s.	v.r.	F pr
Total	530	952.4	1.797		
Treatments	176	392.2	2.229	1.32	0.0176
Genotypes	58	131.2	2.261	1.34	0.0636
Environments	2	6.9	3.445	1.40	0.2479
Block	6	14.8	2.460	1.45	0.1941
Interactions	116	254.2	2.191	1.29	0.0413
IPCA 1	59	179.5	3.043	1.80	<0.001
IPCA 2	57	74.7	1.310	0.77	0.8806
Residuals	0	0.0			
Error	322	545.4	1.694		

FIGURES

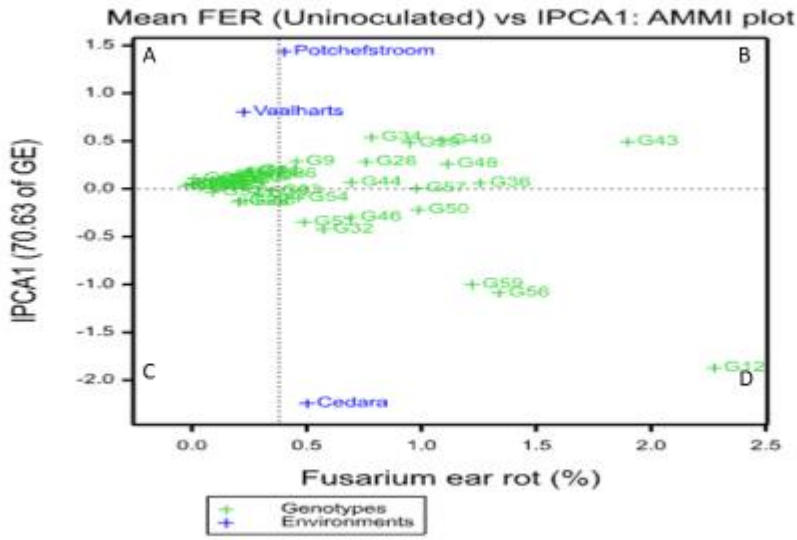

Figure 1 Additive main effects and multiplicative interaction biplot-1 for IPCA 1 scores of 59 cultivars in 3 environments against mean Fusarium ear rot (%) for both genotypes and environments. Genotype/cultivar names are listed in the Appendix A.

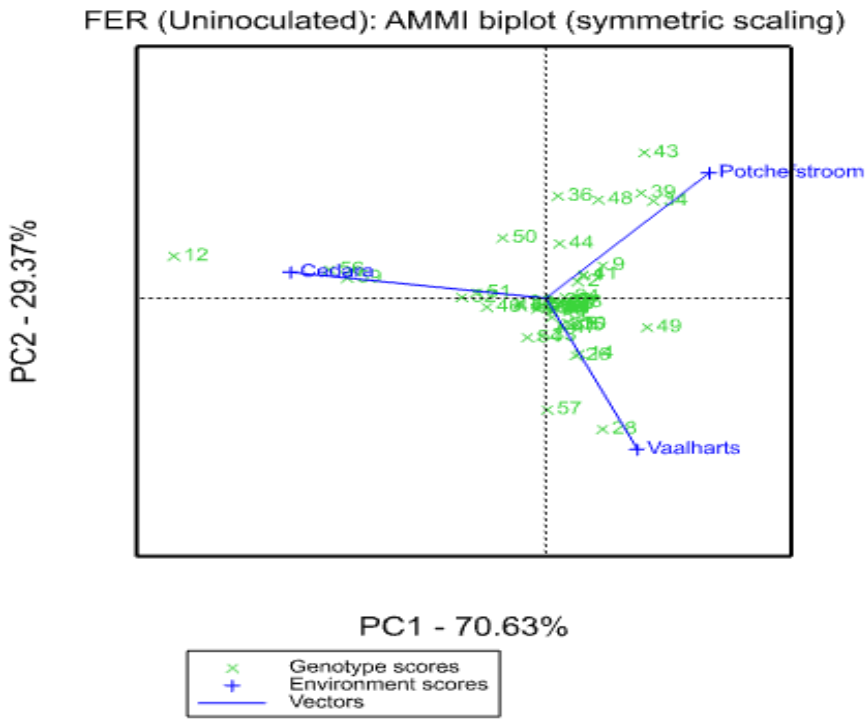

Figure 2 Additive main effects and multiplicative interaction biplot-2 for IPCA 1 vs IPCA 2 scores of 59 cultivars in 3 environments for both genotypes and environments. Genotype/cultivar names are listed in the Appendix A.

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