THE MAIZE TRUST

FINAL REPORT FOR COMPLETED MYCOTOXIN RESEARCH PROJECTS

Project no 14-07

Closing date :	**March 2019**

1. Title of research project

Development of fumonisin resistant germplasm for inclusion into public and commercial breeding programs

2. Personal details *(refer to application)*

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4. Project duration and funding overview *(refer to application)*

Duration of project:	Start date (month, year) **April 2015** End date (month, year**) March 2019**
Total budget of project:	R1 200 000
Amount received from Maize Trust:	R600 000

Other sources of funding for this project:	Contributor ARC	Amount received R600 000

5. Summary *(description of the project, capturing the main findings; maximum 250 words)*

A trial of elite lines from the ARC-Grain Crops and from IITA, Cimmyt germplasm bank were bulked and stored prior to being planted. These were planted at two localities (Potchefstroom and Makhatini) with one replicate per locality to screen for fumonisin resistance. These lines were planted with a row spacing of 1 m with intra row spacing of 30 cm. Each row was 5 m long. Rows were inoculated with a mixture of four high fumonisin producing *F. verticillioides* isolates, mixed in equal proportions (MRC 826, GCI340, GCI51 and GCI1608). At harvest grain samples were collected from the two field trials of the 393 elite lines and were milled. The milled samples were quantified for fumonisin levels using LCMS at the facility at Stellenbosch University. Lines differed in resistance to fumonisin production and ranged from 0.02 to 39.95 ug/kg.

A trial consisting of the four resistant lines (CML 444, CB 248, CB 290, CB 222), each crossed with 50 randomly selected elite lines was planted in Makhathini in June 2017 in a nursery to increase seed. Test cross hybrids were evaluated at Potchefstroom during the 2017/18 season using a random block design with three replications. Hybrids were inoculated at silk stage using a spore suspension of four high fumonisin producing *F. verticillioides* isolates (MRC 826, GCI340, GCI51 and GCI1608), mixed in equal proportions. The trial was planted with a row spacing of 1 m with intra row spacing of 30 cm. Each row was 10 m long. At harvest grain samples were collected and milled and stored at -20 ^{o}C. Samples were then removed and quantified for fumonisin levels using HPLC. The four resistant lines differed significantly one another for fumonisin resistance with CML444 being more susceptible than CB290 and CB222. The crosses did not differ significantly from each other.

6. Objectives *(refer to application)*

6.1 Strategic objectives *(alignment with Maize Trust objectives)*

processing].

Strategic objectives	Yes/N
To support the establishment of the magnitude of mycotoxin contamination of maize during the stages of its production, storage, and processing in South Africa.	No

To support the regular monitoring of the occurrence of the fumonisins, aflatoxins, zearalenone, and trichothecenes (DON and NIV) in locally produced and imported maize.	No	
To support the determination of the factors which contribute to mycotoxin contamination during the production (pre-harvest), storage (post-harvest) and processing of maize.	No	
To support the development of practical, affordable and environmentally sound methods to manage toxigenic fungi in maize, with particular emphasis on the introduction of resistance in local maize cultivars.	Yes	
To support the development of sound mycotoxin risk management practices in the maize supply chain to ensure the delivery of safe products to the consumer.	Yes	

6.2. **Project objectives** (List main objectives)

This section is to be supported by substantial scientific information and key references (maximum 2000 words)

Fusarium verticillioides is a widespread pathogen resulting in Fusarium ear rot (FER) and it can produce fumonisins, which may cause mycotoxicoses in humans and animals. The development of FER and fumonisin resistant cultivars will be a primary strategy in an integrated disease management system. Resistance is an economical and efficient method in reducing FER and fumonisin accumulation in maize and will benefit the whole industry. To achieve the development of resistant cultivars, conventional and transgenic breeding strategies need to be combined. This will include concepts developed in recent projects to develop FER and fumonisin resistant genotypes, agronomically suited to local conditions. Previous collaborative studies (Stellenbosch University, ARC-GCI, CIMMYT, Nairobi University) identified resistant sources in lines CML 444, CB 290, CB 248 and CB 222. This indicated that fumonisin resistance is a reality and can be improved in maize hybrids. Previous studies have focused only on FER symptom development which may not necessarily be related to fumonisin contamination. The proposed study will be the first resistance breeding program in the world that include FER and fumonisins as separate events. This project will be integrated with present collaborative projects.

The following specific objectives have been defined for the proposed project:

- To screen 430 elite lines over 2 localities/seasons to identify and compare new sources of stable resistance to FER and fumonisins. This database will be made available to all breeding programs.

- To test hybrids in development using lines characterized in objective 1 to identify fumonisin resistance in hybrid combinations using CML 444, CB 290, CB 248 and CB 222 as primary resistance sources. Further selections can be made of genotypes with required good agronomic traits and fumonisin resistance for future breeding programs.

6.2 Project objectives *(list main objectives)*

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- To test hybrids in development using lines characterized in objective 1 to identify fumonisin resistance in hybrid combinations using CML 444, CB 290, CB 248 and CB 222 as primary resistance sources. Further selections can be made of genotypes with required good agronomic traits and fumonisin resistance for future breeding programs.
- Diallele analysis to determine inheritance of fumonisin resistance.

7. Work plan *(refer to application)*

7.1 Work plan as stated in the application *(list main tasks)*

<u>Screening of elite inbred lines for resistance to fumonisins</u>

In previous studies (MT and CGIAR funded), progress has been made in screening a limited number of lines at a time with some overlap. The studies enabled the determination of the variance between maize lines in terms of fumonisin resistance and included the refinement of screening procedures. This study entailed the screening of 430 selected

elite lines from the national germplasm bank and other sources (IITA, CIMMYT). Firstly, only small quantities were made available and seed had to be increased in the first season. Annually, 430 lines, including 4 checks were screened (Only 1 replicate) over 2 localities (Potchefstroom and Makhatini). Lines were inoculated using a spore suspension of four high fumonisin producing isolates, mixed in equal proportions (MRC 826, GCI340, GCI51 and GCI1608). Plants were inoculated at silking using a syringe, fumonisin levels will be quantified using LCMS.

Test cross hybrid development and evaluation

Selected elite lines will be crossed to CML 444, CB 290, CB 248 and CB 222 as primary resistance sources. These crosses were made at Makhatini in a season, however there were some issues with pollination as not all lines synchronized. The following season at Potchefstroom some of these crosses were carried out taking time of flowering into account. Test cross hybrids were evaluated at Potchefstroom using a random block design with three replications. Hybrids will be inoculated and fumonisins quantified using HPLC.

Timeframe:

2nd Quarter (Apr - June 2015)

- Harvest first 430 lines that were increased at Potchefstroom, and thresh trial entries.
- Submit biannual report (Oct 2012 - Mar 2013) to the Maize Trust.

3rd Quarter (Jul - Sep 2015)

- Mill samples collected.
  Plan next season's entries for test crosses and screening of 430 lines at Potchefstroom and Makhatini.
  Plant the first screening trial at Makhatini for 430 lines

4th Quarter (Oct - Dec 2014)

- Plant test cross and hybrid screening trials.
  - Maintenance of field trials.
  - Submit biannual report (April - Sept 2013) to the Maize Trust.

1st Quarter (Jan - Apr 2015)

• Inoculate test crosses and line screening trials. • Assist with maintenance of field trials. 2015-2016: Harvest Makhatini lines and thresh, Plant the Potchefstroom trial for screening 430 lines, make crosses at Potchefstroom 2016-2017: Analyse samples of lines screened at Makhatini and Potchefstroom using LCMS, Complete making crosses at Potchefstroom. 2017-2018: Complete data capture of lines screened, Plant crosses at Potchefstroom. 2018-2019 Harvest crosses, thresh and analyse samples using HPLC.

7.2. Achieved tasks according to the stated work plan *(list measurable units as milestones and provide an indication of progress made, e.g. tasks achieved or not [add additional rows if necessary])*

Milestones	**Achievements**
Screening of lines	Mycotoxin analyses and quantification of fumonisin-producing *Fusarium* fungi completed. Samples were analysed using LCMS analysis at Stellenbosch University
Test cross hybrid evaluation	A trial consisting of four resistant lines (CML 444, CB 248, CB 290 and CB 222), each crossed with 50 susceptible lines was planted in Makhatini to increase seed. Seed from the crosses and parent lines were harvested and planted in Potchefsroom in a random block design with three replicates. These were inoculated as stated above and harvested and milled. HPLC analysis was used to quantify fumonisins.

8. Detailed report covering the research during the full grant period *(introduction, methods, results, tables, figures and discussion)*

Introduction

South Africa is the largest maize producer in the Southern African Development Community (SADC), with an average annual production of approximately 11.8 million tons p.a. between 2012 and 2016 (FAO 2018). Over 9000 commercial producers contribute primarily to this production, although small-scale farmers also contribute (Grain 2014).

South Africa frequently has significant maize surpluses for export to neighboring countries (DAFF 2013), however, adverse growing conditions, variable environmental conditions and destructive pests and pathogens threaten the sustainable cultivation of maize in South Africa. This raises serious concerns regarding food security in the SADC.

One of the important microorganisms associated with maize in South Africa is the fungus *Fusarium verticillioides* (Sacc.) Nirenberg. This pathogen is associated with the crop in all maize growing regions of the world. It is best known for causing Fusarium ear rot (FER), but disease symptoms can range from asymptomatic infection to severe rotting of all plant parts (Munkvold, 2003). Fusarium ear rot occurs under warm and dry environmental conditions and affects commercial, emerging and subsistence farmers, resulting in reduced grain quality and yield losses (Marin, *et al*. 1999, White 1999, Munkvold 2003). More concerning is the production of fumonisins that possess cancer-promoting properties (IARC 2002).

Fumonisins have been related to oesophageal cancer in rural areas in South Africa (Rheeder *et al*. 1992; IARC 2002) and have been implicated in human birth defects (Marasas *et al*. 2004). Fumonisins further cause leukoencephalomalacia in equine animals (Kellerman *et al*. 1990), pulmonary oedema in pigs (Harrison et al. 1990) and immuno-suppression in chickens (Marijanovic *et al*. 1991; Qureshi and Hagler 1992). Since fumonisins can be found in both symptomatic and asymptomatic kernels, minimising fumonisin accumulation in maize has become a priority in food safety research. The widespread incidence of fumonisins in maize and maize-based products intended for human and animal consumption have resulted in a number of countries introducing maximum tolerable levels for the fumonisins (Bolger *et al*. 2001; NGFA 2011). Grain produced commercially, and in certain rural areas in South Africa, has been observed with fumonisin levels in excess of South African maximum tolerable limits (4000 µg/kg for processed maize; 2000 µg/kg for human consumption) (Shephard *et al*. 2007; Shephard 2008; Ncube *et al*. 2011; Boutigny *et al*. 2012; Janse van Rensburg *et al*. 2015; Government Gazette of South Africa 2016). Due to the high fumonisin levels detected in South African grain, management methods are concentrated on improving host resistance. Locally adapted breeding material and tropical germplasm has recently been assessed for resistance to FER and fumonisin contamination (Small *et al*. 2012; Rose *et al*. 2016, 2017). These lines and crosses could be utilised in a resistance-breeding programme for the development of resistance varieties.

The planting of resistant cultivars, as part of an integrated disease management strategy, is regarded as an economical and effective approach to reduce FER and minimise the risk of fumonisin accumulation in maize (Harrison *et al*. 1990; Munkvold and Desjardins 1997; Schjøth *et al*. 2008). Sources with good resistance are needed for the development of cultivars resistant to *F. verticillioides* and fumonisin contamination (King and Scott 1981). However, increased screening of lines and crosses of elite lines to resistant sources is still essential in the development of resistant breeding material and cultivars.

It is, therefore, crucial to develop maize cultivars with broad adaptability that are stable in their expression of disease resistance. Alternatively, breeding material and hybrids should be evaluated in multi-environment trials to determine new sources of resistance. This

involves the identification of resistant inbred lines for breeding purposes by evaluating such lines. The aim of this study, therefore, was to evaluate 430 lines and F1 hybrids derived from fumonisin- resistant inbred lines, previously identified in South Africa, and crossed with elite lines for improved resistance to FER and fumonisin accumulation in South Africa. The performance of each genotype was measured for fumonisin contamination, no yield data was recorded.

Material and methods

Maize inbred lines previously characterised for their response to FER and fumonisin accumulation (Small *et al*. 2012; Rose *et al*. 2016, 2017) and previously identified as elite lines used in ARC-GCI breeding programs (Table 1) were used in this study. In the first study 430 lines were screened and in the second study F1 hybrids were generated (Table 2) by crossing each of 4 resistant sources to 50 elite lines. The 430 elite lines were field evaluated at two locations in South Africa; namely Potchefstroom (grid reference: 26°730S, 27°070E; altitude, 1 349 m.a.s.l.) and Makhatini (grid reference: 22°390S, 32°170E; altitude, 77 m.a.s.l.). Potchefstroom is in a dry and warm region, while Makhatini is in a high humidity hot region of the South African maize-production area. The 430 inbred lines were initially planted to bulk up adequate seed for screening and to make the necessary crosses. The 430 inbred lines were screened initially at Makhatini where a single replicate screening trial was planted with 1m inter and 30cm intra row spacing's. This was then repeated in a single replicate trial at Potchefstroom with 1.2 m inter row and 30 cm intra row spacing's. Field trials at Potchefstroom were conducted under dryland conditions where moisture stress was monitored and overhead irrigation performed when necessary. The Makhatini trials were conducted under overhead irrigation and watered twice weekly. Standard maize field practices were used at each locality to fertilise and keep trials free of weeds. Trials were harvested at approximately 12 % grain moisture and stored at -4^{o}C until fumonisin analysis using LCMS at Stellenbosch University could be carried out.

Table 1: Four hundred and thirty elite lines planted in Makhathini and Potchefstroom

CB 334	CB 250	CB 295	CB 406	CBY 323LM-1822
CB 321	CB 255	CB 399	CB 407	CBY 313LM-1812
CB 343	CB 310	CB 197	CB 410	CBY 376LM-1875
CB 318	CB 301	CB 198	CB 125	CBY 377LM-1876
CB246	CB 312	CB 393	CBY 008LM-1507	CBY 394LM-1893
CBY 291	CB 213	CB 220	CBY 011LM-1510	CB 235
CBY 222	CB 221	CB 378	CBY 017LM-1516	CB 343
CBY 202	CB 219	CB 391	CBY 020LM-1519	CB 302
CBY 201	CB 300	CB 392	CBY 022LM-1521	CB 322
CBY 182	CB 307	CB 330	CBY 024LM-1523	CB 221

CBY 175	CB 311	CB 353	CBY 025LM-1524	CB 222
CBY 171	CB 315	CB 356	CBY 032LM-1531	CB 236
CBY 168	CB 316	CB 360	CBY 030LM-1529	CB 248
CBY 166	CB 317	CB 365	CBY 033LM-1837	CB 260
CBY 163	CB 238	CB 366	CBY 038LM-1537	CB 293
CBY 162	CB 249	CB 379	CBY 040LM-1539	CB 305
CBY 157	CB 256	CB 305	CBY 041LM-1540	CB 309
CBY 001	CB 200	CB 354	CBY 043LM-1542	CB 318
CBY 23	CB 209	CB 355	CBY 044LM-1543	CB 327
CBY 14	CB 215	CB 358	CBY 046LM-1545	CB 331
CBY 8	CB 223	CB 359	CBY 050LM-1549	CB 344
CBY 24	CB 232	CB 364	CBY 047LM-1546	CB 389
CBY 45	CB 247	CB 374	CBY 051LM-1550	CBY 004LM-1503
CBY 81	CB 234	CB 323	CBY 052LM-1551	CBY 007LM-1506
CBY 101	CB 319	CB 338	CBY 053LM-1552	CBY 009LM-1508
CBY 113	CB 320	CB 347	CBY 054LM-1553	CBY 013LM-1512
CBY 122	CB 333	CB 381	CBY 057LM-1556	CBY CBYLM-1511
CBY 149	CB 336	CB 382	CBY 060LM-1559	CBY015LM-1514
CB 299	CB 337	CB 384	CBY 063LM-1562	CBY 023LM-1522
CB 302	CB 339	CB 386	CBY 064LM-1563	CBY 059LM-1558
CB 313	CB 341	CB 388	CBY 065LM-1564	CBY 087LM-1586
CB 258	CB 344	CB 394	CBY 066LM-1565	CBY 089LM-1588
CB 340	CB 349	CB 395	CBY 067LM-1566	CBY 092LM-1591
CB 235	CB 350	CB 396	CBY 068LM-1567	CBY 096LM-1595
CB 308	CB 352	CB 397	CBY 069LM-1568	CBY 005LM-1504
CB 237	CB 306	CB 398	CBY 071LM-1570	CBY 062LM-1561
CB 244	CB 373	CB 400	CBY 072LM-1571	CBY 104LM-1603
CB 239	CB 390	CB 404	CBY 074LM-1573	CBY 110LM-1609
CB 236	CB 394	CB 405	CBY 075LM-1574	CBY 114LM-1613
CBY 082LM1581	CBY 208LM-1707	CBY 306LM-1805	CED 69	CBY 113LM-1612
CBY 099LM-1598	CBY211LM-1710	CBY 319LM-1818	CED 83-CN00/122-2	CBY 127LM-1626
CBY 079LM-1578	CBY 216LM-1715	CBY 322LM-1821	CED 081	CBY 140LM-1639
CBY 081LM-1580	CBY218LM-1717	CBY 325LM-1824	CED 90	CBY 149LM-1648
CBY 080LM-1579	CBY 221LM-1720	CBY 326LM-1825	CED 102	CBY 131LM-1630
CBY 095LM-1594	CBY 233LM-1732	CBY 331LM-1830	CED 109	CBY 143LM-1642
CBY 097LM-1596	CBY 236LM-1735	CBY 332LM-1831	CED 142	CBY 121LM-1620

CBY 098LM-1597	CBY 237LM-1736	CBY 333LM-1832	CED 143	CBY 146LM-1645
CBY 101LM-1600	CBY 242LM-1741	CBY 334LM-1833	CED 144	CBY 156LM-1655
CBY 102LM-1601	CBY 243LM-1742	CBY 335LM-1834	CED 150	CBY 210LM-1709
CBY 103LM-1602	CBY 246LM-1745	CBY 337LM-1836	CED 167	CBY 134LM-1633
CBY 104LM-1604	CBY 247LM-1746	CBY 338LM-1837	CED 168	CBY 281LM-1780
CBY 106LM-1605	CBY 245LM-1744	CBY 340LM-1839	CED 173	CBY 283LM-1782
CBY 108LM-1607	CBY 248LM-1747	CBY 347LM-1846	CED 209	CBY 299LM-1798
CBY 109LM-1608	CBY 249LM-1748	CBY 353LM-1852	CED 212	CED 31
CBY 112LM-1611	CBY 249LM-1753	CBY 358LM-1857	CED 217	CED 38
CBY 115LM-1614	CBY 258LM-1757	CBY 362LM-1861	CED 101	CED 078
CBY 116LM-1615	CBY 271LM-1770	CBY 371LM-1870	CED 005	CED 091
CBY 122LM-1621	CBY 273LM-1772	CBY 374LM-1873	CED 105	CED 114
CBY 124LM-1623	CBY 275LM-1774	CBY 375LM-1874	CED 110	CED 142
CBY 125LM-1624	CBY 279LM-1778	CBY 399LM-1898	CED 112	CED 172
CBY 129LM-1628	CBY 298LM-1797	CBY 400LM-1899	CED 201	CED 174
CBY 154LM-1653	CBY 004	CBY 401LM-1900	CED 69	CED 210
CBY 155LM-1654	CBY 25	CBY 403LM-1902	CED 207	CBY 073LM-1572
CBY 159LM-1658	CBY 056LM-1555	CBY 406LM-1905	CED 208	CBY 076LM-1575
CBY 161LM-1660	CBY 100LM-1593	CBY 407LM-1906	CED 222	CBY 077LM-1576
CBY163LM-1662	CBY 111LM-1610	CBY 415LM-1914	CED 223	CBY 079LM-1578
CBY 164LM-1663	CBY 117LM-1616	CBY 416LM-1915	CED 224	
CBY 165LM-1664	CBY 123LM-1622	CBY 419LM-1918	CED 225	
CBY 166LM-1665	CBY 133LM-1632	CBY 355LM-1854	CED 227	
CBY 169LM-1668	CBY136LM-1635	CBY 327LM-1826	CED 231	
CBY 170LM-1669	CBY 139LM-1638	CED 4	CBY 014LM-1513	
CBY 172LM-1671	CBY 144LM-1643	CED 8	CBY 020LM-1819	
CBY 173LM-1672	CBY 148LM-1647	CED 22	CBY 082LM-1581	
CBY 174LM-1673	CBY 157LM-1656	CED 29	CBY 093LM-1592	
CBY 175LM-1674	CBY 158LM-1657	CED 030	CBY 132LM-1631	
CBY 177LM-1676	CBY 203	CED 033	CBY 201LM-1700	
CBY 180LM-1679	CBY 210LM-1709	CED 035	CBY 203LM-1702	
CBY 181LM-1680	CBY 219LM-1918	CED 037	CBY 207LM-1706	
CBY 182LM-1681	CBY 223LM-1722	CED 005	CBY 215LM-1714	
CBY 184LM-1683	CBY 234LM-1733	CED 008	CBY 235LM-1734	
CBY 190LM-1689	CBY 241LM-1740	CED 074	CBY 244LM-1734	
CBY 192LM-1691	CBY 272LM-1771	CED 077	CBY 269LM-1768	

CBY 200LM-1699	CBY 277LM-1776	CED 67	CBY 280LM-1779	
CBY 205LM-1704	CBY298LM-1795	CED 68	CBY 311LM-1810	
CBY 206LM-1705	CBY 301LM-1800	CED 94	CED 323	

Table 2: List of 50 susceptible lines which were crossed with four resistant lines

Lines to cross									
1	CB258	11	CBY24	21	CB250	31	CB237	41	CBY258
2	CB299	12	GP367	22	CBY216	32	CBY044	42	CB236
3	CB246	13	CBY103	23	CBY102	33	CB223	43	CBY122
4	CB192	14	CBY163	24	CB197	34	CB238	44	CB302
5	CBY243	15	CB244	25	CBY333	35	CB320	45	CB220
6	CB209	16	CBY248	26	CB301	36	CB308	46	CBY184
7	CB319	17	CBY374	27	CB200	37	CB198	47	CB300
8	CB215	18	CB249	28	CB255	38	CB315	48	CB235
9	CB232	19	CBY331	29	CB239	39	CB219	49	CBY326
10	CB182	20	CBY169	30	CB249	40	CB256	50	CB310
Four resistant Lines									
1	CML444								
2	CB248								
3	CB290								
4	CB222								

The parental resistant inbred lines (CML 444, CB 248, CB 290 and CB 222) were crossed, at both Makhatini and Potchefstroom to make up adequately bulked seed, with 50 elite lines to make F1 hybrids which were planted in a randomised complete block design with three replications per entry at Potchefstroom. Field trials at Potchefstroom and Makhatini were conducted as explained above. Seed was similarly stored at -4^{o}C until fumonisins could be quantified using HPLC.

Artificial inoculation and grain evaluation

Maize ears in both the line screening and screening of F1 crosses were artificially inoculated with *F. verticillioides* isolates mixed in equal proportions (MRC 826, GCI340, GCI51 and GCI1608) according to Small et al. (2012). Maize plants were left to dry in the field, followed by hand-harvesting of each experimental plot, when grain reached 12 % moisture content. Ears were shelled and grain samples were stored as previously mentioned. When space was available to do fumonisin analysis a sample (250 g) was processed to flour until fumonisin extractions were performed.

Fumonisin analysis of the lines study

Fumonisin B^1, B^2 and B^3 levels in milled grain were determined by liquid chromatography tandem mass spectrometry analysis according to Rose et al. (2016, 2017). Ninety-five percent pure standards of fumonisin B^1 (10 mg), B^2 (10 mg), and B^3 (1 mg), were obtained from the Medical Research Council- Programme on Mycotoxins and Experimental Carcinogenesis, South Africa. Calibration standards ranged between 50 and 2000 µg/kg (FB^1 and FB^2) and between 5 and 2000 µg/kg for FB3. The minimum limit of detection was 20 µg/kg, 0.002 µg/kg and 0.02 µg/kg for FB^1, FB^2 and FB^3, respectively. Samples were analysed by the Central Analytical Facility (Mass spectrometry unit), Stellenbosch University.

Fumonisin analysis of the F1 cross study

Fumonisins were analysed using the HPLC-VICAM method (Janse van Rensburg *et al*., 2015). Fumonisin standards were obtained from CPUT. To generate a standard curve, standards were evaporated and reconstituted with a calibration standard solution ranging from 310 to 5000 µg/kg. Fluorescence was performed at excitation and emission wavelengths of 335 nm and 440 nm respectively using a Waters 2475 multi λ fluorescence detector equipped with a Symmetry C18 (5 µm 3.9 x 150 mm) analytical column (Waters, Milford, USA). The LOD of the method used was 16 µg/kg and R^2 values were ≥99%. Total fumonisins were determined as the sum of FB1+FB2+FB3.

Data analysis

The line screening was replicated by planting one rep at Potchefstroom and one rep at Makhatini due to budget restrictions as THRIP funding was not approved for this study. Reliance on THRIP funding for the completion of this study would have enabled the investigators in this study to do more replicates. Lines were ranked accordingly from most resistant to most susceptible.

Data transformations (Log 10) were performed as needed and evaluated using the Shapiro–Wilk test for normality on the lines and the cross evaluations. The total fumonisins concentration were Log 10 transformed to obtain a normal distribution. The factorial analysis of variance (ANOVA) was performed on the transformed data to determine the differences between crosses and the 4 resistant lines used for the crosses using Genstat. Fisher's least significant difference (LSD) test was calculated at the 95% confidence level.

Results

Screening of elite lines for resistance to fumonisins

Table 3 shows the significant differences of 393 elite maize lines screened for resistance to fumonisins with a range, from most resistant to most susceptible, of 0.02 µg/kg (CBY 005) to 39.95 µg/kg (CBY 139LM-1638). Pearsons ranking correlations between the two

localities were not significant (Correlation coefficient of 0.025). The mean fumonisin level at Makhatini (2.83 µg/kg) was significantly greater than Potchefstroom (0.98 µg/kg).

Table 3 Significant differences of maize lines screened for resistance to fumonisins

	Makhatini	Potchefstroom		
Lines	Total fumonisins µg/kg	Total fumonisins µg/kg	Mean fumonisins µg/kg	Differences
CBY 005	0.03	0.01	0.02	i
CB 334	0.01	0.04	0.03	i
CB 337	0.06	0.03	0.05	i
CB 344	0.02	0.07	0.05	i
CBY 109LM-1608	0.05	0.05	0.05	i
CBY 182	0.09	0.02	0.06	i
CBY 079LM-1578	0.08	0.04	0.06	i
CBY 273LM-1772	0.06	0.07	0.07	i
CBY 067LM-1566	0.05	0.1	0.08	i
CB 239	0.14	0.02	0.08	i
CB 341	0.09	0.07	0.08	i
CBY 291	0.1	0.06	0.08	i
CBY 113	0.04	0.14	0.09	i
CB 355	0.07	0.12	0.10	i
CBY 123LM-1622	0.05	0.14	0.10	i
CB 219	0.1	0.1	0.10	i
CBY 149	0.07	0.13	0.10	i
CBY 201	0.09	0.11	0.10	i
CBY 272LM-1771	0.05	0.15	0.10	i
CED 035	0.11	0.1	0.11	i
CB 410	0.11	0.11	0.11	i
CBY 353LM-1852	0.15	0.07	0.11	i
CB 394	0.17	0.06	0.12	i
CB 393	0.13	0.11	0.12	i
CBY 122	0.16	0.08	0.12	i
CBY 376LM-1875	0.15	0.09	0.12	i
CB 395	0.07	0.17	0.12	i
CB 313	0.19	0.06	0.13	i
CBY 033LM-1837	0.15	0.1	0.13	i
CBY 103LM-1602	0.23	0.03	0.13	i
CB 339	0.24	0.04	0.14	i
CBY 101	0.14	0.17	0.16	i
CBY 331LM-1830	0.13	0.19	0.16	i
CB 364	0.1	0.22	0.16	i
CBY 322LM-1821	0.26	0.07	0.17	i
CB 305	0.22	0.12	0.17	i

CBY 111LM-1610	0.05	0.29	0.17	i
CBY 076LM-1575	0.17	0.18	0.18	i
CBY 243LM-1742	0.16	0.19	0.18	i
CBY 180LM-1679	0.33	0.03	0.18	i
CB 336	0.11	0.26	0.19	i
CB 373	0.18	0.19	0.19	i
CBY 025LM-1524	0.03	0.34	0.19	i
CBY 158LM-1657	0.3	0.08	0.19	i
CBY 246LM-1745	0.04	0.35	0.20	i
CBY 165LM-1664	0.32	0.07	0.20	i
CB 255	0.18	0.22	0.20	i
CB 249	0.37	0.04	0.21	i
CBY 401LM-1900	0.11	0.3	0.21	i
CBY 046LM-1545	0.08	0.33	0.21	i
CBY 258LM-1757	0.32	0.09	0.21	i
CBY 115LM-1614	0.09	0.34	0.22	i
CBY 011LM-1510	0.2	0.24	0.22	i
CBY218LM-1717	0.37	0.07	0.22	i
CBY 064LM-1563	0.17	0.27	0.22	i
CB 378	0.4	0.06	0.23	i
CB 299	0.11	0.36	0.24	i
CBY 074LM-1573	0.08	0.39	0.24	i
CBY 45	0.45	0.02	0.24	i
CBY 166	0.39	0.09	0.24	i
CED 4	0.27	0.21	0.24	i
CB 301	0.44	0.05	0.25	i
CB 316	0.45	0.04	0.25	i
CED 22	0.47	0.02	0.25	i
CB 209	0.28	0.22	0.25	i
CBY 24	0.31	0.19	0.25	i
CB 237	0.47	0.05	0.26	i
CB 302	0.43	0.1	0.27	i
CBY 047LM-1546	0.14	0.39	0.27	i
CBY 222	0.5	0.03	0.27	i
CBY 313LM-1812	0.39	0.14	0.27	i
CBY 133LM-1632	0.07	0.47	0.27	i
CED 227	0.29	0.25	0.27	i
CBY 235LM-1734	0.48	0.07	0.28	i
CBY 311LM-1810	0.54	0.01	0.28	i
CB 356	0.29	0.27	0.28	i
CBY136LM-1635	0.54	0.03	0.29	i
CED 69	0.26	0.31	0.29	i
CBY 004	0.27	0.31	0.29	i
CBY 208LM-1707	0.45	0.13	0.29	i

CB 312	0.42	0.19	0.31	i
CBY 053LM-1552	0.46	0.15	0.31	i
CED 31	0.49	0.13	0.31	i
CED 008	0.09	0.54	0.32	i
CBY 190LM-1689	0.48	0.16	0.32	i
CBY 275LM-1774	0.63	0.02	0.33	i
CBY 338LM-1837	0.15	0.5	0.33	i
CBY 169LM-1668	0.45	0.23	0.34	i
CBY 241LM-1740	0.15	0.54	0.35	i
CBY 419LM-1918	0.62	0.08	0.35	i
CBY 143LM-1642	0.67	0.04	0.36	i
CED 209	0.49	0.22	0.36	i
CB 338	0.44	0.28	0.36	i
CBY 277LM-1776	0.4	0.32	0.36	i
CBY 052LM-1551	0.68	0.06	0.37	i
CBY 041LM-1540	0.08	0.71	0.40	i
CED 208	0.27	0.52	0.40	i
CB 359	0.14	0.66	0.40	i
CBY 056LM-1555	0.55	0.25	0.40	i
CBY 099LM-1598	0.73	0.07	0.40	i
CB 236	0.76	0.05	0.41	i
CBY 100LM-1593	0.49	0.32	0.41	i
CBY 206LM-1705	0.8	0.01	0.41	i
CBY 001	0.75	0.07	0.41	i
CB 223	0.33	0.51	0.42	i
CED 005	0.51	0.35	0.43	i
CED 223	0.77	0.09	0.43	i
CB 215	0.58	0.29	0.44	i
CBY 098LM-1597	0.77	0.1	0.44	i
CB 311	0.83	0.05	0.44	i
CBY 071LM-1570	0.85	0.03	0.44	i
CBY 202	0.32	0.57	0.45	i
CB 300	0.85	0.04	0.45	i
CBY 050LM-1549	0.88	0.02	0.45	i
CB 322	0.81	0.1	0.46	i
CED 201	0.79	0.13	0.46	i
CBY 14	0.47	0.46	0.47	i
CBY 051LM-1550	0.12	0.81	0.47	i
CBY 205LM-1704	0.84	0.1	0.47	i
CED 94	0.71	0.23	0.47	i
CB 308	0.87	0.08	0.48	i
CB 333	0.26	0.69	0.48	i
CBY 080LM-1579	0.61	0.34	0.48	i
CBY 125LM-1624	0.86	0.09	0.48	i

CED 172	0.84	0.12	0.48	i
CB 384	0.84	0.12	0.48	i
CB 340	0.05	0.93	0.49	i
CB 404	0.42	0.57	0.50	i
CED 224	0.29	0.7	0.50	i
CB 386	0.17	0.83	0.50	i
CBY 040LM-1539	0.37	0.64	0.51	i
CED 033	0.78	0.24	0.51	i
CBY 416LM-1915	0.82	0.21	0.52	i
CBY 097LM-1596	0.96	0.08	0.52	i
CBY 146LM-1645	0.87	0.17	0.52	i
CED 144	0.54	0.5	0.52	i
CBY 223LM-1722	0.16	0.89	0.53	i
CBY 175	0.97	0.09	0.53	i
CED 8	1.03	0.03	0.53	i
CB 295	0.53	0.54	0.54	i
CB 256	1.03	0.09	0.56	i
CBY 245LM-1744	0.62	0.53	0.58	i
CED 142	1.11	0.05	0.58	i
CBY 140LM-1639	1.02	0.17	0.60	i
CBY 116LM-1615	1.18	0.02	0.60	i
CBY 166LM-1665	0.1	1.1	0.60	i
CB 310	0.13	1.08	0.61	i
CBY 332LM-1831	1.16	0.05	0.61	i
CBY 406LM-1905	0.07	1.14	0.61	i
CBY 095LM-1594	1.06	0.17	0.62	i
CBY 400LM-1899	1.13	0.1	0.62	i
CBY 134LM-1633	1.18	0.07	0.63	i
CBY 024LM-1523	1.18	0.07	0.63	i
CBY 210LM-1709	1.21	0.04	0.63	i
CBY 127LM-1626	1.22	0.05	0.64	i
CB 397	1.13	0.14	0.64	i
CBY 066LM-1565	1.09	0.18	0.64	i
CBY 108LM-1607	0.75	0.52	0.64	i
CB 315	1.27	0.02	0.65	i
CBY 247LM-1746	0.85	0.44	0.65	i
CB 365	1.24	0.06	0.65	i
CBY 301LM-1800	1.22	0.08	0.65	i
CB 306	1.07	0.24	0.66	i
CB 343	0.2	1.11	0.66	i
CED 207	1.23	0.1	0.67	i
CED 38	1.2	0.13	0.67	i
CBY 340LM-1839	1.26	0.08	0.67	i
CB 321	1.32	0.04	0.68	i

CB 319	0.76	0.62	0.69	i
CB 396	1.27	0.11	0.69	i
CBY 082LM-1581	0.91	0.49	0.70	i
CBY 203LM-1702	0.26	1.15	0.71	i
CB 221	1.37	0.06	0.72	i
CBY 219LM-1918	1.12	0.32	0.72	i
CBY 81	1.01	0.48	0.75	i
CBY 065LM-1564	0.7	0.8	0.75	i
CB 307	0.44	1.07	0.76	i
CED 074	0.8	0.73	0.77	i
CBY 157	1.12	0.43	0.78	i
CBY 233LM-1732	1.54	0.02	0.78	i
CED 077	1.29	0.28	0.79	i
CBY 174LM-1673	1.26	0.32	0.79	i
CBY 043LM-1542	1.49	0.11	0.80	i
CBY 022LM-1521	1.36	0.26	0.81	i
CBY 394LM-1893	1.55	0.07	0.81	i
CED 167	1.6	0.02	0.81	i
CB 220	1.54	0.12	0.83	i
CBY 129LM-1628	1.52	0.14	0.83	i
CBY 8	0.47	1.21	0.84	i
CBY 038LM-1537	0.48	1.21	0.85	i
CBY 102LM-1601	1.64	0.07	0.86	i
CBY 327LM-1826	1.63	0.08	0.86	i
CBY 082LM1581	1.04	0.71	0.88	i
CBY 269LM-1768	0.66	1.09	0.88	i
CBY 207LM-1706	1.7	0.07	0.89	i
CBY 399LM-1898	0.7	1.09	0.90	i
CED 168	1.76	0.04	0.90	i
CBY 159LM-1658	1.47	0.34	0.91	i
CBY 184LM-1683	0.59	1.23	0.91	i
CBY 089LM-1588	1.82	0.03	0.93	i
CBY 203	1.8	0.05	0.93	i
CBY 075LM-1574	1.7	0.16	0.93	i
CB 347	1.65	0.22	0.94	i
CB 198	1.8	0.07	0.94	i
CBY 122LM-1621	1.82	0.1	0.96	i
CB 222	1.89	0.04	0.97	i
CBY 156LM-1655	1.93	0.02	0.98	i
CBY 168	1.73	0.22	0.98	i
CBY 104LM-1604	1.54	0.42	0.98	i
CB 360	1.34	0.63	0.99	i
CBY 362LM-1861	1.93	0.05	0.99	i
CBY 106LM-1605	1.92	0.08	1.00	i

CBY 371LM-1870	0.83	1.18	1.01	i
CBY 004LM-1503	2	0.04	1.02	i
CBY 069LM-1568	1.92	0.12	1.02	i
CED 174	1.4	0.66	1.03	i
CBY 407LM-1906	1.47	0.63	1.05	i
CBY 131LM-1630	2.03	0.13	1.08	i
CBY 323LM-1822	2.09	0.09	1.09	i
CBY163LM-1662	1.85	0.38	1.12	i
CB 381	2.16	0.08	1.12	i
CBY 110LM-1609	2.17	0.08	1.13	i
CB 320	2.21	0.06	1.14	i
CB 354	1.95	0.33	1.14	i
CB 244	2.08	0.2	1.14	i
CBY 215LM-1714	2.05	0.25	1.15	i
CBY 009LM-1508	2.2	0.11	1.16	i
CBY 155LM-1654	1.55	0.76	1.16	i
CB 258	2.22	0.14	1.18	hi
CBY 326LM-1825	1.69	0.71	1.20	ghi
CBY 121LM-1620	1.43	1	1.22	ghi
CBY 374LM-1873	2.02	0.41	1.22	ghi
CED 150	2.34	0.14	1.24	ghi
CBY 298LM-1797	1.22	1.27	1.25	ghi
CBY 25	0.91	1.59	1.25	ghi
CB 405	2.42	0.09	1.26	ghi
CB 382	2.31	0.2	1.26	ghi
CED 037	2.44	0.08	1.26	ghi
CB 293	1.82	0.71	1.27	ghi
CBY 092LM-1591	2.53	0.01	1.27	ghi
CBY 182LM-1681	0.91	1.63	1.27	ghi
CBY 112LM-1611	2.01	0.58	1.30	ghi
CBY 279LM-1778	2.6	0.01	1.31	ghi
CBY 337LM-1836	1.36	1.25	1.31	ghi
CBY 216LM-1715	1.93	0.7	1.32	ghi
CBY 173LM-1672	2.56	0.09	1.33	ghi
CB 213	0.44	2.24	1.34	ghi
CBY 333LM-1832	2.16	0.52	1.34	ghi
CB 323	1.25	1.44	1.35	ghi
CBY 149LM-1648	2.69	0.03	1.36	ghi
CBY 177LM-1676	2.06	0.67	1.37	ghi
CBY 358LM-1857	2.12	0.61	1.37	ghi
CBY 007LM-1506	2.76	0.06	1.41	ghi
CBY 403LM-1902	0.09	2.74	1.42	ghi
CBY 057LM-1556	0.92	1.92	1.42	ghi
CBY 104LM-1603	2.44	0.41	1.43	ghi

CED 143	2.47	0.4	1.44	ghi
CED 078	1.69	1.2	1.45	ghi
CB 327	2.55	0.35	1.45	ghi
CBY 283LM-1782	2.84	0.06	1.45	ghi
CB 391	2.61	0.3	1.46	ghi
CBY 172LM-1671	2.73	0.18	1.46	ghi
CED 210	2.73	0.2	1.47	ghi
CBY 242LM-1741	2.26	0.7	1.48	ghi
CBY 096LM-1595	3.02	0.01	1.52	fghi
CBY 073LM-1572	2.94	0.11	1.53	fghi
CBY 234LM-1733	2.97	0.08	1.53	fghi
CB 388	0.16	2.93	1.55	fghi
CBY 375LM-1874	2.84	0.26	1.55	fghi
CB 407	2.21	0.91	1.56	fghi
CBY 154LM-1653	2.76	0.37	1.57	fghi
CED 67	3.09	0.04	1.57	fghi
CBY 201LM-1700	2.75	0.4	1.58	fghi
CB 309	3.2	0.05	1.63	fghi
CBY 068LM-1567	3.22	0.09	1.66	fghi
CB 235	3.27	0.05	1.66	fghi
CBY 236LM-1735	3.12	0.2	1.66	fghi
CBY 299LM-1798	3.13	0.2	1.67	fghi
CB 358	3.25	0.1	1.68	fghi
CBY 017LM-1516	2.45	0.93	1.69	fghi
CBY 306LM-1805	2.86	0.56	1.71	fghi
CBY 020LM-1519	3.32	0.12	1.72	fghi
CBY 132LM-1631	3.44	0.08	1.76	fghi
CBY 175LM-1674	2.37	1.15	1.76	fghi
CBY 072LM-1571	3.43	0.12	1.78	fghi
CBY298LM-1795	0.32	3.32	1.82	fghi
CED 112	3.58	0.07	1.83	fghi
CED 091	3.49	0.17	1.83	fghi
CBY 355LM-1854	2.29	1.39	1.84	fghi
CBY 013LM-1512	3.26	0.43	1.85	fghi
CBY 271LM-1770	3.24	0.5	1.87	fghi
CB 389	3.36	0.46	1.91	fghi
CB 374	3.55	0.28	1.92	fghi
CB 398	3.78	0.06	1.92	fghi
CB 406	0.29	3.57	1.93	fghi
CB 379	3.8	0.11	1.96	fghi
CBY 281LM-1780	0.51	3.41	1.96	fghi
CB 318	3.86	0.08	1.97	fghi
CBY 077LM-1576	0.12	3.91	2.02	fghi
CBY 144LM-1643	1.6	2.44	2.02	fghi

CBY 335LM-1834	4.06	0.1	2.08	fghi
CBY 059LM-1558	4.17	0.08	2.13	fghi
CBY 334LM-1833	4.1	0.21	2.16	fghi
CBY 248LM-1747	3.81	0.55	2.18	fghi
CBY 249LM-1748	0.24	4.12	2.18	fghi
CBY 087LM-1586	4.46	0.04	2.25	fghi
CED 102	1.2	3.36	2.28	fghi
CBY 044LM-1543	1.36	3.26	2.31	fghi
CB 248	1.79	2.85	2.32	fghi
CBY 377LM-1876	2.26	2.52	2.39	fghi
CBY 117LM-1616	3.79	1.01	2.40	fghi
CBY 005LM-1504	4.82	0.04	2.43	fghi
CBY 020LM-1819	4.65	0.28	2.47	fghi
CBY 054LM-1553	0.13	4.82	2.48	fghi
CB 247	4.46	0.51	2.49	fghi
CB 366	4.32	0.66	2.49	fghi
CBY 237LM-1736	3.64	1.37	2.51	fghi
CBY 030LM-1529	4.44	0.57	2.51	fghi
CBY 032LM-1531	0.02	5.01	2.52	fghi
CED 231	4.83	0.49	2.66	fghi
CBY 171	1.6	3.82	2.71	fghi
CBY015LM-1514	2.51	2.93	2.72	fghi
CBY 023LM-1522	1.84	3.67	2.76	fghi
CB 400	5.38	0.16	2.77	fghi
CED 081	5.56	0.06	2.81	fghi
CB 125	3.73	1.89	2.81	fghi
CBY 347LM-1846	5.61	0.11	2.86	fghi
CBY 415LM-1914	4.86	0.92	2.89	fghi
CBY 114LM-1613	2.73	3.06	2.90	fghi
CBY 23	5.79	0.05	2.92	fghi
CED 29	1.53	4.4	2.97	fghi
CB 349	5.71	0.27	2.99	fghi
CBY 113LM-1612	5.26	0.76	3.01	fghi
CBY211LM-1710	5.1	0.93	3.02	fghi
CED 90	4.79	1.28	3.04	fghi
CBY 164LM-1663	6.05	0.05	3.05	fghi
CBY 081LM-1580	4	2.11	3.06	fghi
CB 330	5.21	0.9	3.06	fghi
CBY 008LM-1507	5.77	0.5	3.14	fghi
CB 350	6.27	0.09	3.18	fghi
CBY 221LM-1720	5	1.37	3.19	fghi
CB 352	0.05	6.36	3.21	fghi
CB 390	5.61	0.87	3.24	fghi
CBY 163	6.47	0.07	3.27	fghi

CB 331	2.35	4.21	3.28	fghi
CED 222	5.83	0.75	3.29	fghi
CB 392	6.72	0.14	3.43	efghi
CED 323	0.44	6.54	3.49	efghi
CED 68	6.29	0.95	3.62	efghi
CBY CBYLM-1511	7.23	0.06	3.65	efghi
CB246	3.26	4.06	3.66	efghi
CB 232	6.62	0.92	3.77	efghi
CBY 060LM-1559	6.83	0.77	3.80	efghi
CB 260	7.55	0.06	3.81	efghi
CBY 170LM-1669	4.17	3.57	3.87	efghi
CBY 062LM-1561	8.02	0.05	4.04	efghi
CBY 200LM-1699	7.92	0.16	4.04	efghi
CB 234	8.07	0.27	4.17	efghi
CBY 244LM-1734	8.55	0.01	4.28	efghi
CBY 148LM-1647	8.74	0.61	4.68	efghi
CBY 319LM-1818	9.31	0.52	4.92	efghi
CBY 181LM-1680	7.07	3.63	5.35	efghi
CED 114	6.69	4.15	5.42	defghi
CBY 063LM-1562	0.11	11.61	5.86	defghi
CBY 192LM-1691	2.31	9.42	5.87	defghi
CED 101	0.75	11.1	5.93	defghi
CBY 161LM-1660	0.37	12.08	6.23	defghi
CED 212	11.62	0.88	6.25	defghi
CED 030	5.5	7.89	6.70	defghi
CB 399	12.09	1.42	6.76	defghi
CED 83-CN00/122-2	13.38	0.28	6.83	defghi
CBY 280LM-1779	14.05	0.05	7.05	defghi
CBY 162	1.42	12.93	7.18	defghi
CB 250	14.1	0.29	7.20	defghi
CB 353	0.54	13.95	7.25	defghi
CED 225	0.69	13.9	7.30	defghi
CBY 124LM-1623	14.13	1.01	7.57	defghi
CBY 157LM-1656	14.02	1.31	7.67	defghi
CBY 249LM-1753	15.14	0.25	7.70	defghi
CBY 325LM-1824	2.13	13.61	7.87	defghi
CED 109	15.69	0.07	7.88	defghi
CED 217	14.14	3.51	8.83	defghi
CBY 093LM-1592	18.93	0.07	9.50	defghi
CB 200	19.67	0.03	9.85	defghi
CED 173	5.49	16.64	11.07	cdefgh
CBY 014LM-1513	9.93	12.24	11.09	cdefg
CB 317	0.27	22.47	11.37	cdef
CB 238	26.34	0.28	13.31	cde

CED 110	27.32	3.2	15.26	bcd
CED 105	35.13	0.16	17.65	bcd
CBY 101LM-1600	47.34	0.1	23.72	b
CB 197	70.29	0.17	35.23	a
CBY 139LM-1638	76.18	3.72	39.95	a

Screening of elite lines crossed with four resistant lines

Table 4 shows the factorial ANOVA analysis of the crosses made from the elite lines to the four resistance sources selected for this study. Very high variation in the replicates resulted in insignificant differences between all the crosses studied. The range of fumonisins in the crosses was from 0.059 to 20.135 µg/kg. This insignificance could mean that the elite lines used are all of similar resistance or natural variation in the trial was very high and therefor the crosses do not differ significantly. However, the main effect of the four resistant lines indicated that the line main effect was significant with the lines CB290 and CB222 being significantly more resistant than CML444 (Table 5).

Table 4. Factorial ANOVA of resistant lines crossed with elite lines screened for fumonisins.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Rep	2	126.0244653	63.0122327	17.63	<.0001
Reslines	3	35.1478395	11.7159465	3.28	0.0211
Crosses	48	151.7437804	3.1613288	0.88	0.6918
Reslines*Crosses	144	357.8085896	2.4847819	0.70	0.9942

Table 5. Resistance to fumonisins in the four resistant parent lines used to cross with elite lines.

Lines	Fumonisins (µg/kg)
CML444	4.134 a
CB248	1.914 ab
CB290	1.252 b
CB222	1.032 b

Discussion

Screening of elite lines

Maize inbred lines with potential resistance to fumonisin accumulation, caused by *F. verticillioides* were identified from well adapted breeding lines used in South Africa. This is of great significance, because *F. verticillioides* is known to be the most common fungal pathogen associated with Fusarium ear rot in the country and because existing cultivars are not known to have sufficient resistance to fumonisin contamination (Rheeder *et al*, 1990). None of the inbred lines in this study were completely resistant to fumonisin

production which is in agreement with Small *et al* (2012). However, some lines had consistently lower fumonisins at Potchefstroom and Makhatini. These lines can be potential sources of resistance to fumonisin production and could be used in a maize breeding program. However, there were many lines that did not differ significantly from the most resistant lines identified in the study. These most resistant lines would need to be screened again under a much higher fumonisin pressures over a number of localities to be able to select the most resistant and stable lines to be used in a breeding program.

The insignificant ranking of the maize lines screened over the two localities (Potchefstroom and Makhatini) may be due to different disease potentials as explained by Flett and McLaren (1994). This would have to be studied by planting different genotypes over a number of localities and seasons to determine the stability of resistance over different disease potentials.

Screening of elite lines crossed with four resistant lines

The four resistant lines used in this study differed significantly with CB290 and CB222 being the most resistant (Small *et al,* 2012; Netshifhefhe *et al*, 2018). However unlike results obtained by Netshifhefhe *et al* (2018) the crosses did not differ significantly. High replicate significant differences and generally low fumonisin levels may explain this anomaly. The fact that the resistant lines used in this study indicates that resistance to fumonisin does occur and could be used in maize breeding programs. However, the lack of significance in the crosses does cast some doubt on the efficacy of these lines used in crosses. Netshifhefhe *et al* (2018) however obtained significant differences between crosses with resistance sources which showed the opposite to findings in this present study. Netshifhefhe *et al* (2018) found the principle gene action to be additive in nature for fumonisin production in maize and that resistance was highly heritable, with resistance in the hybrids being similar to that of the parental inbred lines. Generally low levels of fumonisin were observed in this study which has previously been observed to be the case at Potchefstroom. Interactions between the environment and genotypes are well known to significantly affect fumonisin contamination (Munkvold 2003). Netshifhefhe *et al* (2018) reported that environment primarily contributed to FER severity and fumonisin contamination, whereas the genotype more prominently contributed to *F. verticillioides* colonisation. The hybrids in this study, therefor, need to be studied for resistance across locations with higher fumonisin potentials and over several seasons which may indicate significance between these test hybrids. Netshifhefhe *et al* (2018) reported recently that Makhatini is the more favourable locality to screen for fumonisin resistance. This location had a high relative humidity throughout the growing season and was relatively dry; both conditions that are favourable for *F. verticillioides* growth and fumonisin production during grain filling (month 3), which is the plant's most susceptible developmental stage (Miller 1994). The low infection and fumonisin levels observed in Potchefstroom may be due to the high rainfall as *F. verticillioides* is favoured by dry conditions (Miller 1994).

The principal gene action observed by Netshifhefhe *et al* (2018) was additive in nature for fumonisin production in maize. Resistance to fumonisin contamination was highly heritable, with resistance in the hybrids similar to that of their parental inbred lines. Breeding for resistance using inbred lines with a level of resistance to *F.*

verticillioides/fumonisins is recommended and should result in quicker and more efficient development of resistant maize cultivars.

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9. Scientific outputs

9.1 Outputs of project as stated in the application

Expected outputs	**Numbers**	**Achieved (yes/no or n.a.)**	**Nature of output e.g. title of papers, description, etc.**
Scientific papers:	0		
Technical reports:	3		
Databases:			
Procedures/methods:			
Human capacity development:			
Technology transfer:	0		
Other outputs:			

9.2 Reasons for outputs not achieved

10. Successful institutional and inter-institutional collaboration

Researcher	Institution	Role
Dr Lindy Rose	Stellenbosch University	LCMS analysis
Nicolene Cochrane	ARC-Biometry	Data analysis
Dr Belinda J. van Rensburg	ARC-GC	Fumonisin analysis and inoculum production

11. Benefit of the outputs to the maize industry

Resistant lines identified in the study may be used to develop fumonisin resistant hybrids in private and public breeding programs.

12. Progress with regards to human resource development *(e.g. training of post-graduate students in mycotoxin research)*

A potential PhD student Mr Moses Ramusi was identified and started his PhD on this project. After two years and poor progress he resigned and took up employment elsewhere.

13. Statement whether funds were adequate to complete the project

Originally we were relying on THRIP funding as well as Maize Trust/ARC funding to complete this study. THRIP funding was stopped for the duration of this study. This forced us to prioritise and scale down on what was originally planned for this study.

14. Comments *(discuss anything you wish to share with The Maize Trust)*

Firstly, the researchers were relying heavily on NRF THRIP funding which was awarded the previous year before the study began. However, the season that this study began THRIP funding was not approved. The investigators had to then downscale the original plan. This was done by screening one rep each at Makhatini and Potchefstroom instead of three replicates per locality. When making the crosses initially at Makhatini many lines flowered out of sync with the resistant lines. This forced us to make lines the following season at Potchefstroom taking the various flowering dates into account. Originally we intended planting the crosses at two localities namely Makhatini and Potchefstroom but due to lack of THRIP funding and time constraint (regarding duration of the project we were forced to plant at Potchefstroom only). Unfortunately, the fumonisin levels at Potchefstroom were lower than those at Makhatini, as shown by the line screening studies. Ideally this study should have been planted at both localities. Originally we were going to do a diallele analysis in this project but due to financial constraints we had to consolidate resources. The ARC-GCI did however collaborate with Stellenbosch University on a student project which included a diallele analysis. The Potchefstroom trials of this diallele analysis were funded by this project. The diallele study was published as Netshifhefhe, N.E.I., Flett, B.C., Viljoen, A. & Rose, L.J. 2018. Inheritence and genotype by environment analyses of resistance to *Fusarium verticillioides* and fumonisin contamination in maize F1

hybrids. Euphytica 214:235. This was reported in another project by Dr Lindy Rose.

15. Signature of the Project Leader

	Potchefstroom	2/7/2020
Project Leader	**Place**	**Date**

16. Signature of Responsible Authority

ARC-Grain Crops	**Potchefstroom**	**2 July 2020**
Name / Institution	**Place**	**Date**