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Comparative Assessment of Maize Lines Produced by Different Breeding Methods using both Microbiological and Metabolic Profiling Tools

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INTRODUCTION

The increasing requirements for food safety evaluation have led to the development of novel approaches and methodologies to assess the safety of foods by looking at a new integrated risk analysis approach. In this approach one looks at whether the diversification in agricultural production systems and the different breeding approaches used in the production of foods has lead to a diversification in risks. This project is part of a European Union collaborative project where the contribution of CSIR Biosciences includes the evaluation of different South African and European maize lines, using microbiological and mycotoxin analysis, as well as metabolic profiling through NMR (Nuclear Magnetic Resonance) analysis. This study shows the results obtained with South African maize cultivars produced through conventional breeding and biotechnology approaches. It highlights the benefit of using a comparative assessment as a means of exploring profiling methods to differentiate risks associated with plant pathogens, possibly linking them to specific breeding approaches.

MATERIALS AND METHODS

The 26 maize cultivars used in this study are shown in **Table 1**. For the enumeration of *Fusarium verticillioides* 50 kernels were plated on 10 PCNB agar plates using the method of Rabie *et al*[1]. The mycotoxin analysis was done using the ELISA NEOGEN kit for quantification of Fumonosins and Zearale none. The NMR profiling was done using method developed by Manetti *et al.*[2].

Cultivar	Breeding Approach
DKC 80-10 (2)	CONV
DKC 80-12Y (9)	Bt derivative
DKC 63-20 (18)	CONV
CRN 3505 (22)	CONV
DKC 78-15 Bt (38)	Bt derivative
DKC 80-10 (65)	CONV
DKC 80-12Y (66)	Bt derivative
DKC 63-20 (52)	CONV
CRN 3505 (84)	CONV
DKC 78-15 Bt (85)	Bt derivative
DKC 61-24 (50)	CONV
DKC 61-25 (51)	Bt derivative
DKC 63-28R (54)	Roundup ready derivative
DKC 78-35R (86)	Roundup ready derivative
SNK 6326 B (53)	Bt derivative
DKC 80-30R (67)	Roundup ready derivative
SB7855EZN1 (87)	Bt and Roundup ready derivative
DKC 80-10 (104)	CONV
DKC 80-12Y (105)	Bt derivative
DKC 63-20 (99)	CONV
CRN 3505 (117)	CONV
DKC 61-24 (97)	CONV
DKC 61-25 (98)	Bt derivative
DKC 63-28R (100)	Roundup ready derivative
DKC 78-35R (119)	Roundup ready derivative
DKC 78-15 Bt (118)	Bt derivative

Table 1: Maize cultivars

RESULTS

A difference in infection by *F. verticillioides* of the same maize cultivar grown in different locations was observed for cultivars DKC 80-10, DKC 80-12, DKC 63-20 and CRN 3505 (**Figure 1**). Although DKC 80-12 is a Bt cultivar its behaviour was similar to the other 3 cultivars that were produced by conventional breeding. The sample size is very small to be able to draw any conclusions regarding the breeding practices but the difference in location is definitely a factor.

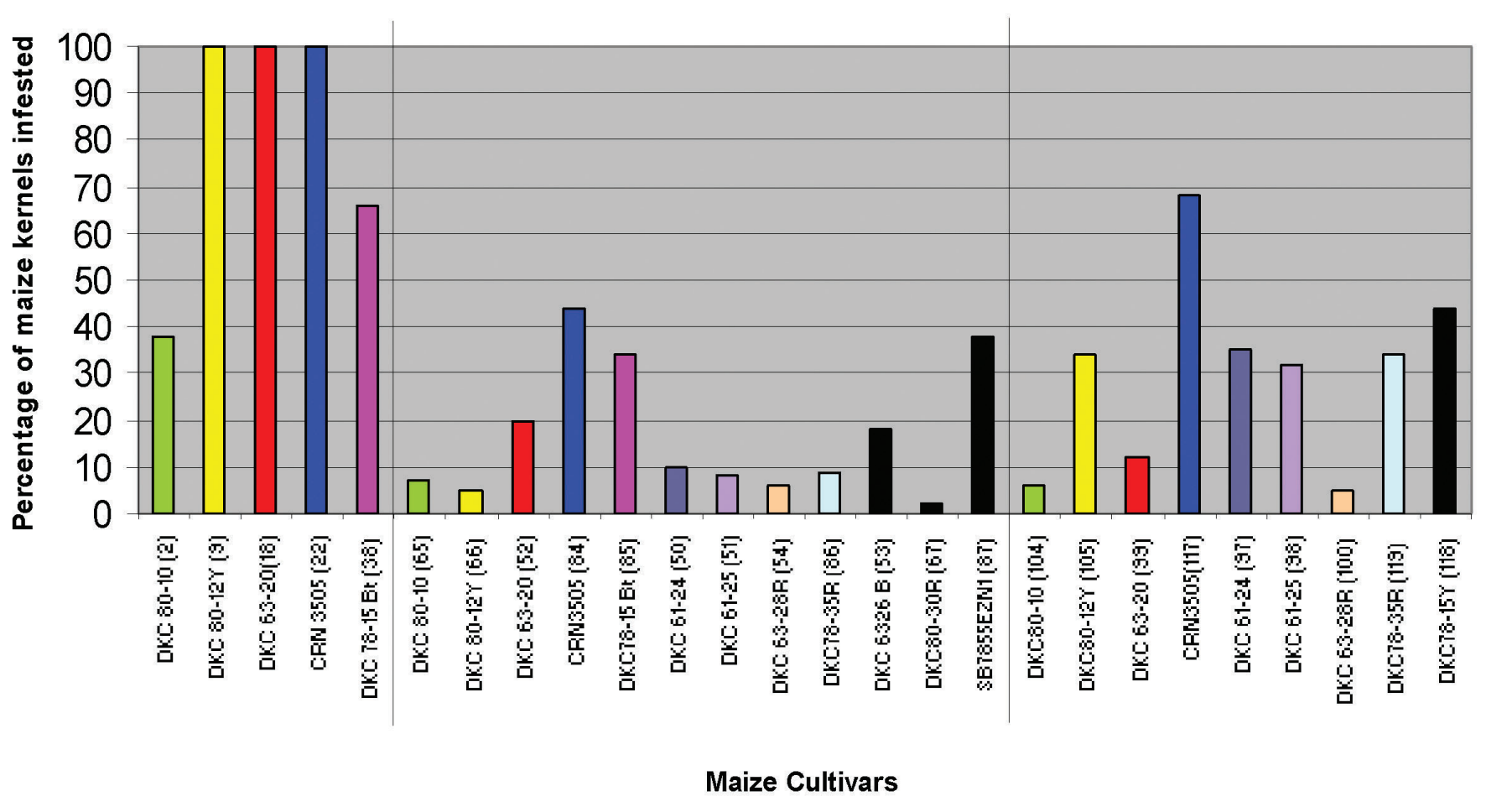

Figure 1: Incidence of *Fusarium verticillioides* in the 26 maize cultivars

The mycotoxin results indicate a greater percentage of fumonosins in the conventionally produced maize in contrast to the GM maize. Again the sample size is too small to draw any definite conclusions.

The NMR spectrum of the maize samples is shown in **Figure 2**. The Principal Component Analysis (PCA) of the maize samples was done by using the high-field regions of the NMR spectra representing amino and organic acid compounds.

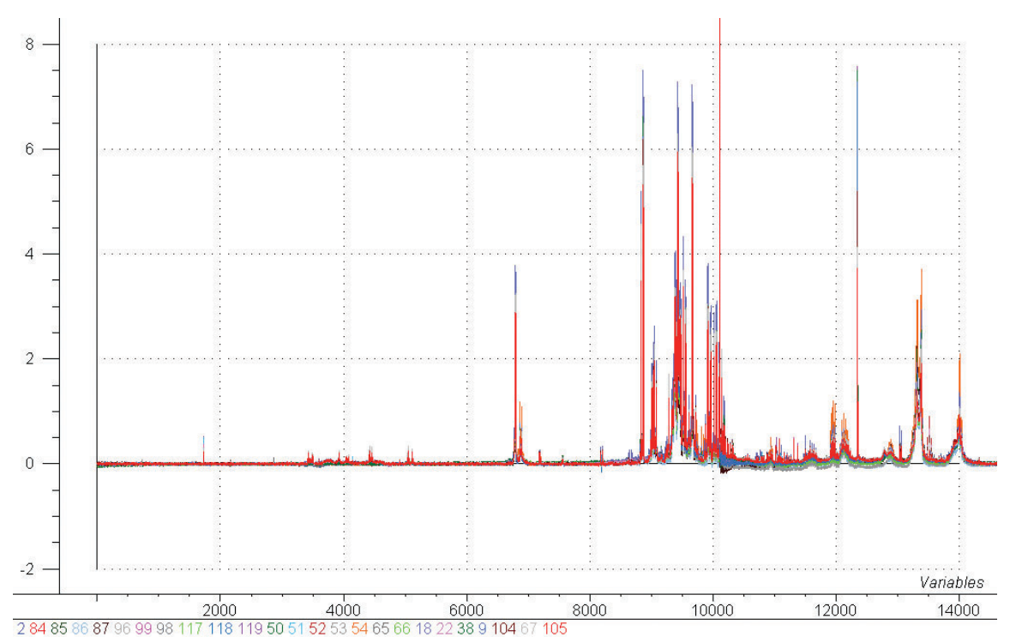

Figure 2: NMR spectrum of maize cultivars

PC2 vs PC3 separated samples by location (**Figure 3**). The maize samples planted in Potchefstroom occupy the top right hand quadrant while the Petit converged to the left side of the graph.

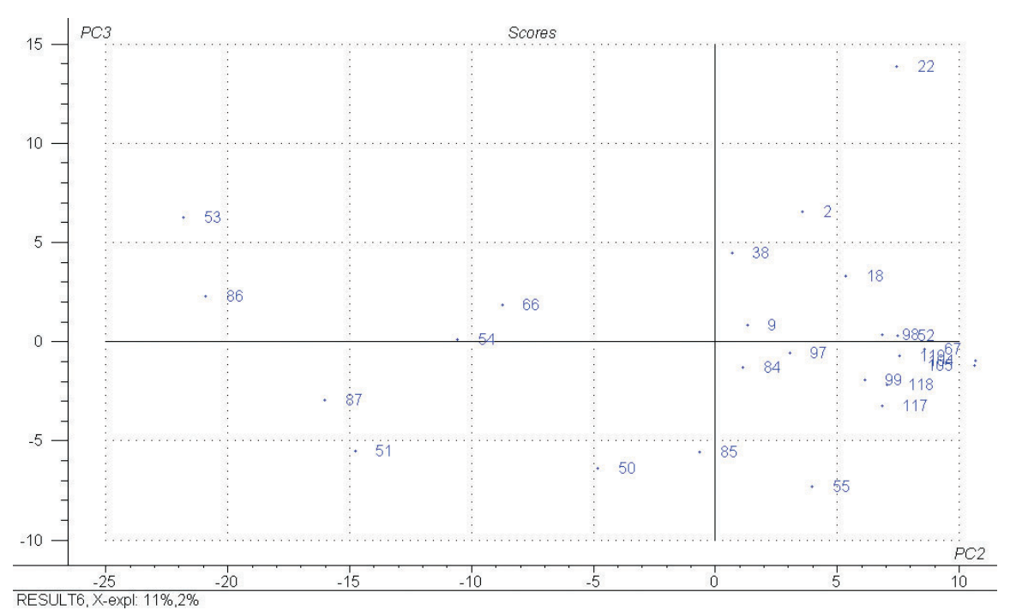

Figure 3: PCA analysis of the maize samples

Breeding System Mycotoxin	Conventional	GMO (Bt)	GMO (RoundUp)	GMO (Bt & RoundUp)
	Positive samples/total analysed samples (%)			
Aflotoxins	0/11 (0%)	0/9 (0%)	0/5 (0%)	0/1 (0%)
Zearalenon	1/11 (9%)	0/9 (0%)	0/5 (0%)	0/1 (0%)
Fumonisin	5/11 (45%)	3/9 (33%)	0/5 (0%)	0/1 (0%)

Table 2: Presence of mycotoxins in maize

CONCLUSIONS

The work presented here refers to South African maize samples that have been analysed for mycotoxins, for presence of *F.verticillioides* and for metabolic profiling. Although the 26 maize samples analysed constitute a very small sample, the results are already indicating location as a source of variation. This is encouraging in terms of stimulating debate on the meaning of such variation and it will be interesting to verify whether this trend will still be there with a much bigger sample size.

Future work will look at more South African samples and will integrate these results with those obtained with the European maize samples. When all the results are integrated, the location where the maize was grown may not play a significant role, whereas the breeding methods or cultivation practices may be the key sources of natural variation. This work will also be complemented with the results obtained with the other technologies like proteomics, microarrays and GC-MS and LC-MS.

REFERENCES

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2. Rabie, CJ; Lübben, A; Marais, GJ; and Jansen van Vuuren, H (1997) Enumeration of fungi in barley. International Journal of Food Microbiology, 35, 117-127