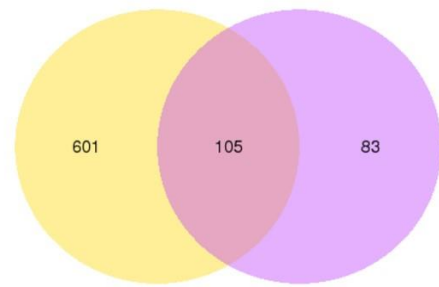


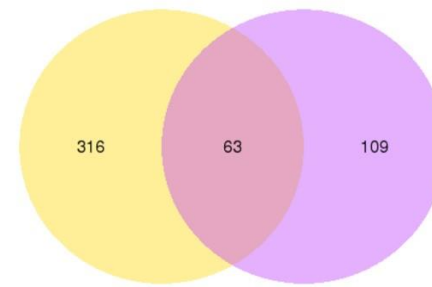
**Figure 1.** Volcano plots inferring the overall distribution of differentially expressed genes ( $q < 0.05$ ) in maize following infection with *Fusarium verticillioides* at (A) 24 hours after inoculation (hai), (B) 48 hai, (C) 72 hai and (D) 7 days after inoculation.



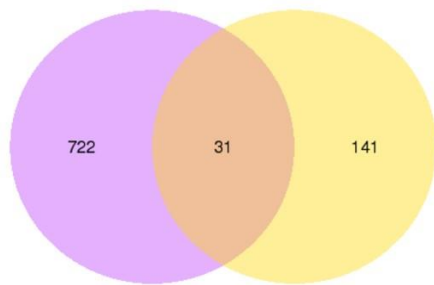
(A)



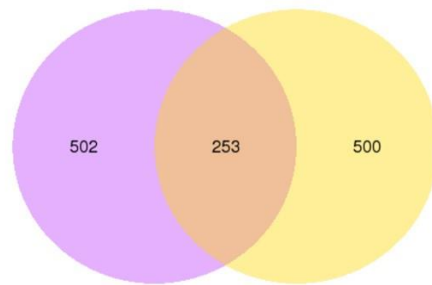
(B)



(C)

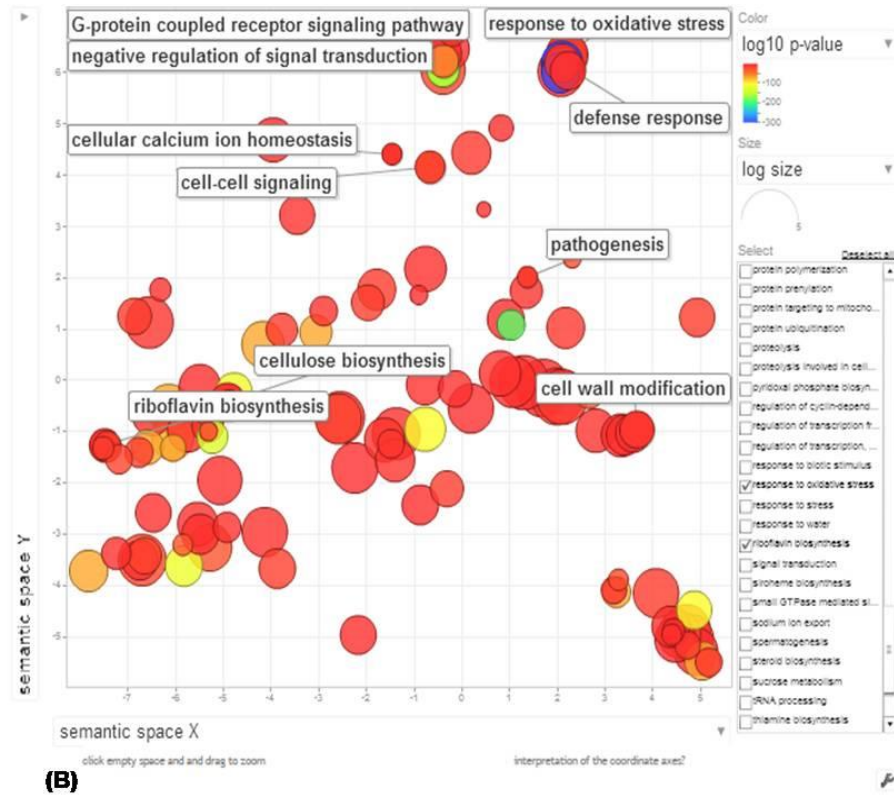
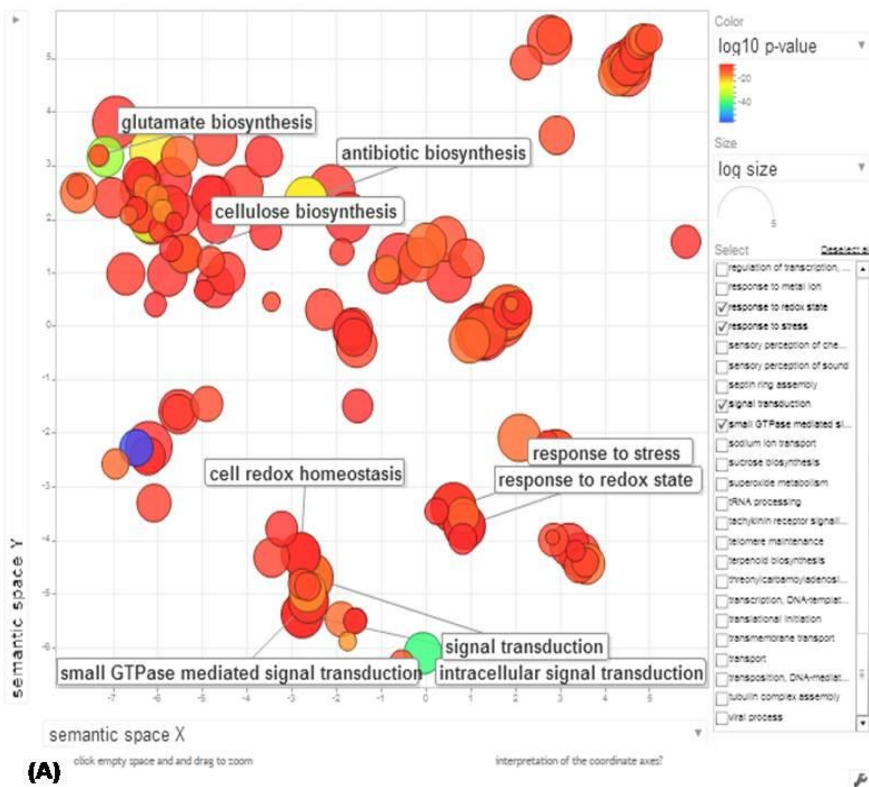


(D)

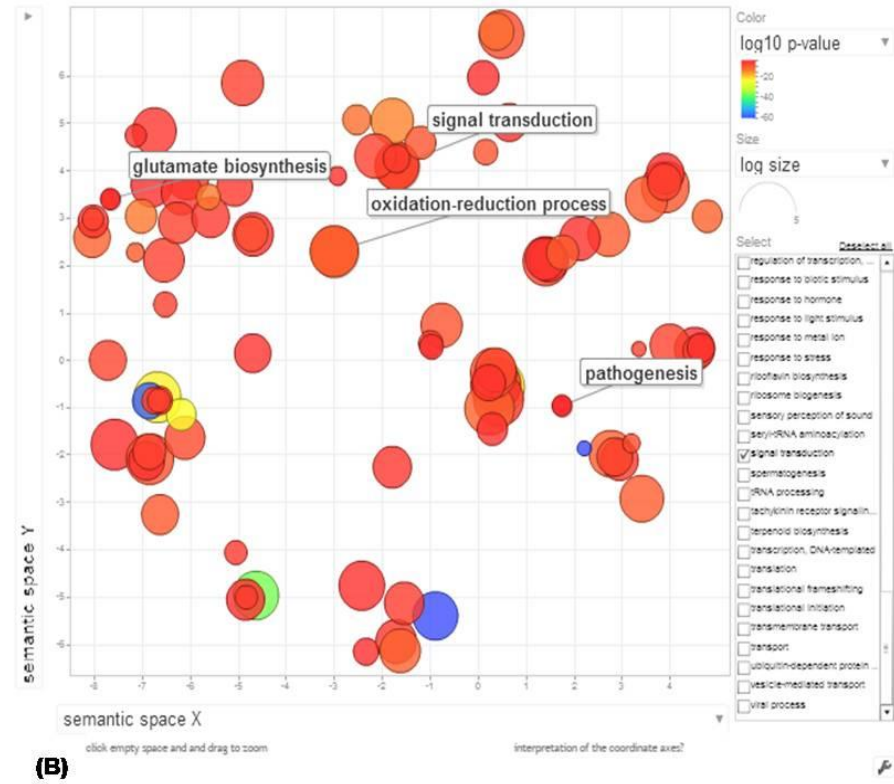
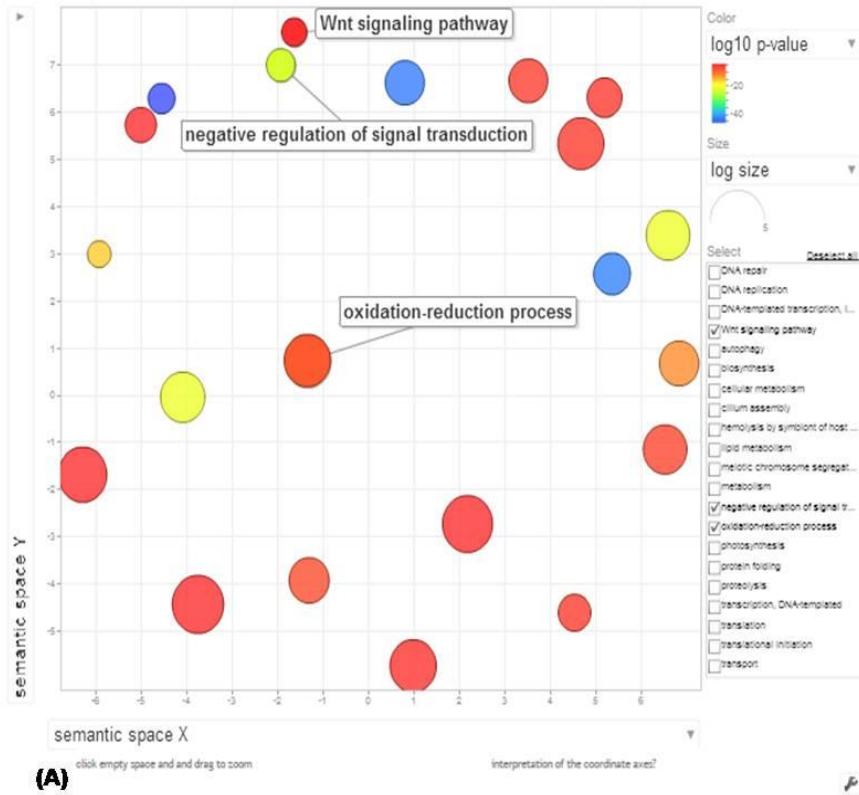


(E)

**Figure 2.** Venn diagrams representing the number of differentially expressed genes following infection of a maize inbred line with *Fusarium verticillioides* at (A) 48 hours after inoculation (hai) vs. 24 hai, (B) 72 vs. 48 hai and (C) 7 days vs. 72 hai.

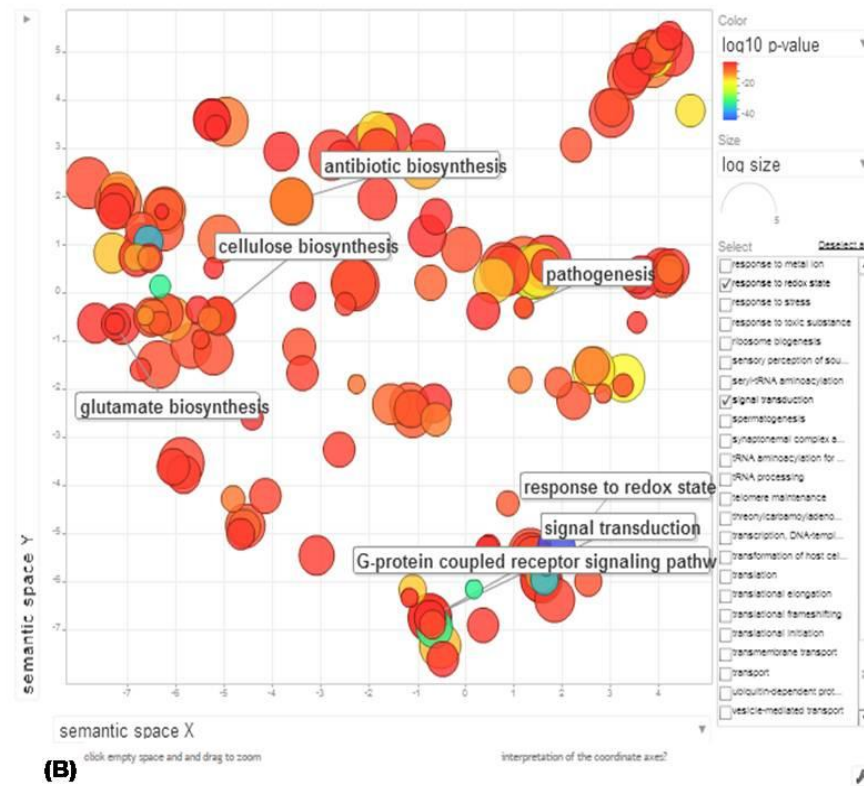
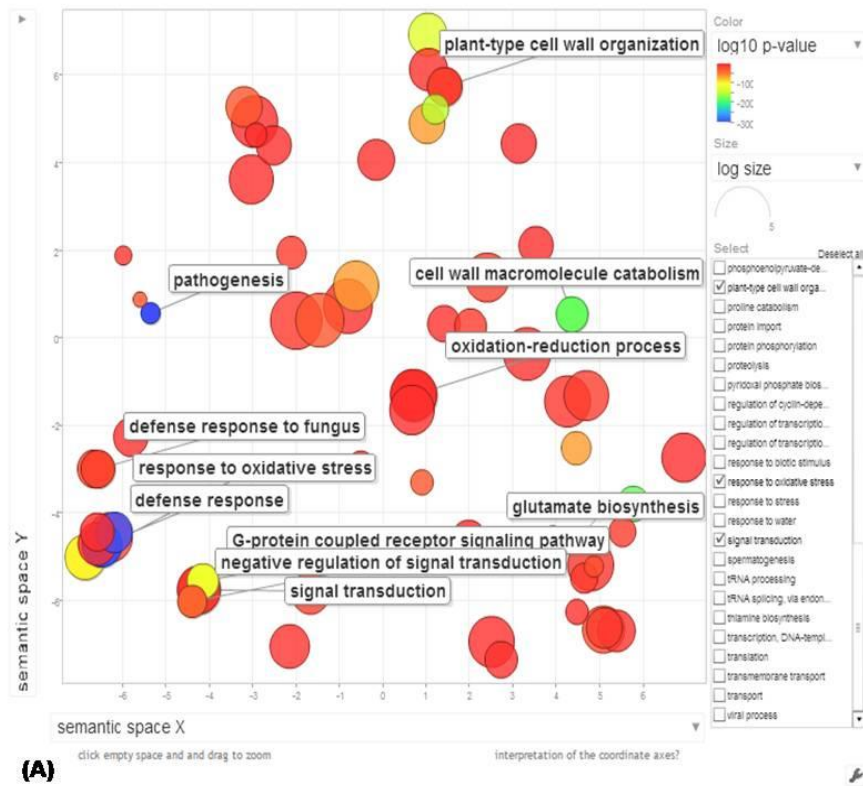


**Figure 3.** Semantic similarity-based Gene Ontology (GO) scatterplots of the differentially expressed genes (A) up-regulated and (B) down-regulated at 24 hours after inoculation.

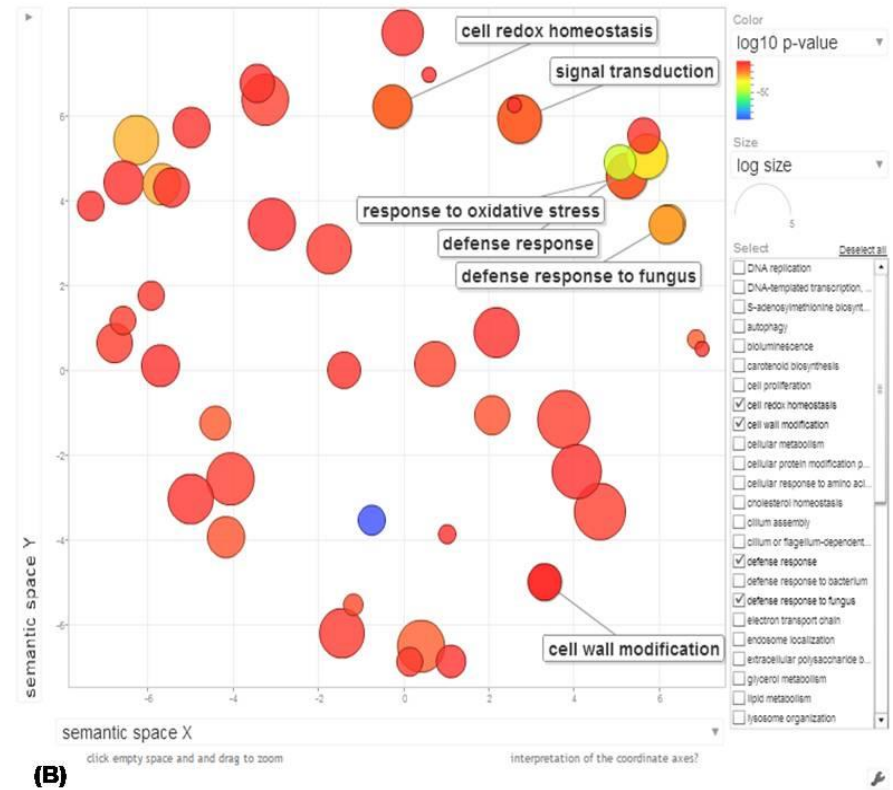
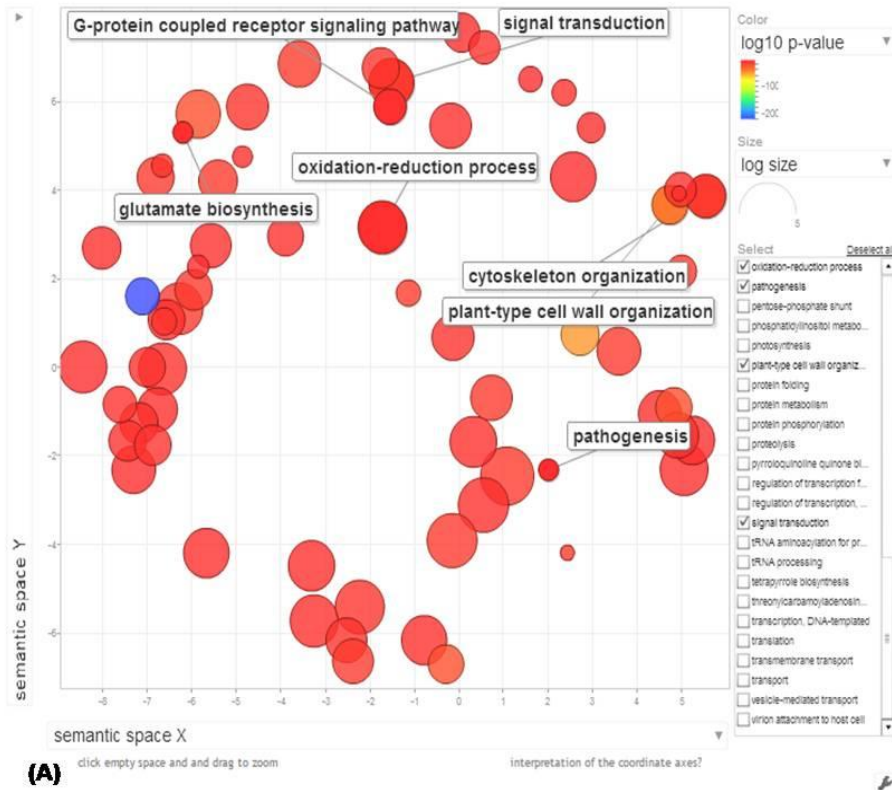


**Figure 4.** Semantic similarity-based Gene Ontology (GO) scatterplots of the differentially expressed genes (A) up-regulated and (B) down-regulated at 48 hours after inoculation.





**Figure 5.** Semantic similarity-based Gene Ontology (GO) scatterplots of the differentially expressed genes (A) up-regulated and (B) down-regulated at 72 hours after inoculation.



**Figure 6.** Semantic similarity-based Gene Ontology (GO) scatterplots of the differentially expressed genes (A) up-regulated and (B) down-regulated at 7 days after inoculation.

## CHAPTER 6

### Conclusion

Maize is an important food and feed crop produced worldwide, and is one of the most important staple foods produced in Africa (Byerlee and Eicher, 1997). The production of maize in Africa, however, is met with numerous constraints, of which ear rot diseases are of significant importance due to food security and food safety issues (Wagacha and Muthomi, 2008). Fusarium ear rot (FER), caused by *Fusarium verticillioides* and Aspergillus ear rot (AER), caused by *Aspergillus flavus*, are considered the most important ear rot diseases in southern and eastern Africa, respectively. In addition, *F. verticillioides* contaminates maize grain with mycotoxins called fumonisins, and *A. flavus* contaminates grain with aflatoxins. The ubiquitous contamination of African-produced maize with mycotoxins can lead to daily intake levels that result in acute and prolonged negative effects on human health. Of the management strategies available to reduce ear rot diseases and mycotoxin contamination of maize grain, host-plant resistance provides the most feasible, environmentally sound and cost-effective means of reducing contamination with mycotoxigenic fungi in the field (Afolabi *et al.*, 2007).

The response of South Africa maize inbred lines to *F. verticillioides* infection and fumonisin accumulation was determined in multiple environments. All maize cultivars produced commercially in the country have insufficient levels of resistance to *F. verticillioides* (Rheeder *et al.*, 1990; Janse van Rensburg *et al.*, 2015), and new FER- and fumonisin-resistant cultivars are thus required. Four inbred lines (CML 390; RO 424W, US 2540W and VO 617y-2) were consistently resistant to the fungus and its toxins across localities over two seasons. They also demonstrated good stability in their response to *F. verticillioides*, confirming their broad-adaptability. The resistant lines can therefore be used as a source of resistance in breeding programmes for the development of new locally-adapted resistant maize hybrids. They also can be of value in research efforts to identify quantitative trait loci for marker-assisted selection, and for discovering genes responsible for resistance for genetic modification of maize.

Kenyan maize inbred lines resistant to *A. flavus* and aflatoxins were evaluated for resistance to *F. verticillioides* and fumonisin accumulation in both South Africa and Kenya. This was the first time the Kenyan material has been screened for resistance to *F. verticillioides* and fumonisins. Several of the Kenyan maize lines outperformed the most resistant South African in both countries. Kenyan inbred line CML 495 was consistently the most resistant to FER and fumonisin contamination, but not significantly more than Kenyan lines CML 264 and CKL05015 and the South African line RO 549W. This study demonstrated that AER/aflatoxin-resistant lines may serve as a source of resistance to *F. verticillioides* and fumonisin accumulation. It also showed that fumonisin contamination of maize grain in Kenya can be severe, as Kiboko in Kenya was found to be the ideal site for FER and fumonisin production in maize grain.

Gamma irradiation is a technology that has become popular for mutation breeding of agricultural crops to generate diversity for increased yields, better agronomic features, and disease resistance. It generates mutants that are stable in a more efficient and publically acceptable way than conventional breeding and genetic modification, respectively. In this study gamma irradiation was used for the first time to mutate elite inbred lines for increased resistance to *F. verticillioides* and fumonisins. M<sub>4</sub> selections derived from the inbred lines proved to be more resistant to FER, *F. verticillioides* colonisation and fumonisin accumulation than their non-irradiated parents. This result provides a basis to study genomic alterations that contribute to enhanced resistance to *F. verticillioides* and fumonisin accumulation by means of advanced biotechnological tools such as next-generation sequencing. The mutants generated in this study still need to be further screened for good agronomic traits (yield, plant height, growth cycle) and general combinability.

The transcriptional response of maize to infection by *F. verticillioides* was studied over a period of 7 days to identify defence-related plant responses and identify genes that could potentially be used for enhanced plant resistance. Transcripts associated with pathogen recognition, signalling molecules, pathogenesis-related genes, cell wall restructuring and secondary hormone-based signalling were induced while *F. verticillioides* colonisation of maize grain continued to increase. The rate of gene expression in maize plants indicated a delayed response based on a pathogen-associated molecular pattern immunity response. Gene expression in resistant and susceptible maize genotypes should be compared in future, and the importance of genes in the defence response confirmed by gene knock-out studies.

This study has provided new information on resistance in maize to *F. verticillioides* and fumonisins. South African maize inbred lines and AER/aflatoxin-resistant maize lines from Kenya have been identified as potential sources of resistance for the development of resistant maize hybrids. The ability of gamma radiation to induce genetic variation for resistance in elite inbred lines has also been demonstrated. Inbreeding of the maize mutants should continue to the M<sub>6</sub>-M<sub>8</sub> generation to ensure homozygosity, and further evaluated in multiple environments to determine broad or specific adaptability for disease resistance. The evaluation of the transcriptional response in maize to *F. verticillioides* 7 days after infection has identified differentially expressed genes that could potentially be used as molecular markers or for genetic engineering to develop maize hybrids resistant to FER and fumonisins.

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