Peanuts that keep aflatoxins at bay: A threshold that matters

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Aflatoxin contamination in peanuts poses major challenges for vulnerable populations in sub-Saharan Africa and South Asia. Developing peanut varieties to combat preharvest *Aspergillus flavus* infection and resulting aflatoxin contamination has thus far remained a major challenge, confounded by a highly complex peanut-*Aspergilli* pathosystem.

Our study reports achieving a high level of resistance in peanuts by overexpressing (OE) antifungal plant defensins MsDef1 and MtDef4,2, and through host-induced gene silencing (HIGS) of aflM and aflP genes from the aflatoxin biosynthetic pathway.

While the former improves genetic resistance to *A. flavus* infection, the latter inhibits aflatoxin production in the event of infection, providing durable resistance against different *Aspergillus flavus* morphotypes and negligible aflatoxin content in several peanut events/lines as well.

A strong positive correlation was observed between aflatoxin accumulation and decline in transcription of the aflatoxin biosynthetic pathway genes in both OE-Def and HIGS lines. Transcriptomic signatures in the resistant lines revealed key mechanisms such as regulation of aflatoxin synthesis, its packaging and export control, besides the role of reactive oxygen species-scavenging enzymes that render enhanced protection in the OE and HIGS lines.

This is the first study to demonstrate highly effective biotechnological strategies for successfully generating peanuts that are near-immune to aflatoxin contamination, offering a panacea for serious food safety, health and trade issues in semi-arid regions.

Contamination by aflatoxins

Aflatoxins, secondary metabolites produced by *Aspergillus flavus* and *A. parasiticus*, are extremely toxic, immunosuppressive and carcinogenic compounds. Over five billion people in developing countries of sub-Saharan

Africa (SSA) and South Asia (SA) are exposed to uncontrolled levels of these toxins, while nearly two billion unsuspectingly consume aflatoxins at levels far above the European standards of 4ppb, especially in low-income countries where food rarely undergoes formal safety inspection.

Alarming levels of aflatoxin contamination in an array of crops, including peanuts, have been reported around the world. Very high levels of aflatoxins B1, B2, G1 and G2 in peanuts, peanut butter and other processed commodities sold in formal and informal markets in low-income countries of SSA and SA are of great concern.

Being a subterranean legume, peanuts are susceptible to contamination from the soil that serves as a reservoir for *Aspergilli*. The developing peanut pods are in direct contact with soil populations of these two aflatoxigenic species that inhabit soils as conidia or sclerotia.

While frequent droughts and high temperatures can cause the pods to shatter – damaging tissues and thereby increasing the chances of preharvest infection – drought adaptation in peanuts is not necessarily linked to the level of resistance to *A. flavus* invasion and aflatoxin accumulation.

Postharvest management

Although postharvest management practices such as appropriate drying, curing and storage can minimise aflatoxin contamination during storage, they can only be effective when peanuts are free from preharvest infection. Biocontrol strategies such as 'competitive atoxigenic' fungal technology (CAFT) and deploying of promiscuous atoxigenic *Aspergillus* strains have been shown to reduce levels of aflatoxin contamination in the field.

Nevertheless, CAFT poses potential challenges in peanuts, as it does not offer protection from exponential mould growth, further compromising peanut quality and hygiene. However, development of varieties with desirable genetic resistance to preharvest infection by *A. flavus* and aflatoxin contamination has remained a challenge for peanut breeding programmes.

Here, we describe a host-plant resistance strategy to create peanut germplasm with improved genetic resistance to *A. flavus* infection and aflatoxin contamination. This is performed using a three-tier approach involving the following:

- Prevention of fungal infection by boosting the innate plant immunity.
- Arrest of subsequent fungal growth in the event of infection.
- Inhibition of aflatoxin production in scenarios where fungal infection is difficult to eradicate.

This approach involved altering interactions of the *Aspergillus*-peanut pathosystem for activation of defence pathways by differentially regulating plant antimicrobial polypeptides (AMPs; defensins) that confer enhanced protection against pathogenic stresses and mechanical wounding and expressing double-stranded RNA molecules of *Aspergillus* in the peanut-host system to inactivate and suppress key aflatoxin biosynthetic pathway genes.

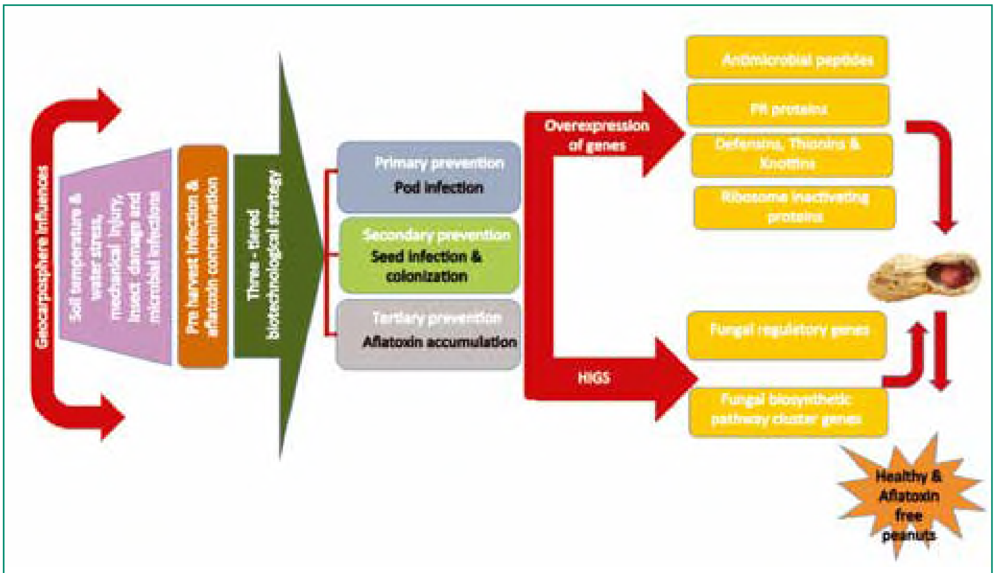
Summary of findings

In summary, our study demonstrated that defensins boost resistance of peanuts against the invading *A. flavus*, providing agronomically useful levels of control, and functional inhibition of the ver-1 (aflM) and omtA (aflP) genes through HIGS results in remarkable resistance to aflatoxin contamination.

Our data shows that by using two different interventions, we achieved aflatoxin levels in peanuts that are nondetectable or as low as 1-2ppb (within the safety limits). This finding is of high significance, as there are no resistant peanut lines/varieties available that demonstrate resistance levels even remotely close to the United States or European Union legislative limitation of <20ppb and <4ppb aflatoxin, respectively.

Data presented here suggests that co-expression of antifungal defensins and hpRNAs targeting mycotoxin genes in transgenic peanuts could boost immunity, potentially resulting in absolute aflatoxin control. As a future follow-up, we propose a strategy for addressing the complex host-*A. flavus* interactions using biotechnological approaches for effective control of preharvest infection and aflatoxin management in peanuts (*Figure 1*).

Figure 1: Biotechnological approaches are necessary for effective control of preharvest infection and aflatoxin management in peanuts.



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