Antifungal plant defensins MtDef4 and MtDef5: Mechanisms of action and engineering transgenic peanut resistant to Aspergillus flavus and aflatoxin accumulation

Dilip Shah[1], Jagdeep Kaur[1], Tariq Islam[1], Siva Velivelli[1], Kiran Sharma[2] and Pooja Bhatnagar[2]

1. Donald Danforth Plant Science Center, Saint Louis, MO 63132
2. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, Telangana, India

Fungal pathogens impose major constraints on crop yields globally. Host defense peptides have evolved in plants to protect from the damaging effects of fungal pathogens. Defensins are sequence divergent cysteine-rich antifungal peptides of innate immunity expressed in all plants. They exhibit potent antifungal activity in vitro and therefore have potential for use in transgenic crops for enhanced resistance to fungal pathogens. MtDef4 and MtDef5 are two sequence-divergent apoplast-localized defensins expressed in Medicago truncatula. MtDef4 is a monomeric defensin of 47 amino acids, whereas MtDef5 is a novel bi-domain defensin containing two monomeric domains linked by a 7-amino acid peptide. These defensins differ from each other in sequence, net charge and hydrophobicity. MtDef4 inhibits the growth of several filamentous fungi including Fusarium graminearum at micromolar concentrations. In contrast, the bi-domain MtDef5 inhibits the growth of these fungi at submicromolar concentrations. Two ascomycete fungi N. crassa and F. graminearum respond differently to MtDef4 challenge[1]. Membrane permeabilization is required for the antifungal activity of MtDef4 against F. graminearum but not against N. crassa. MtDef4 is internalized by these fungi, but is targeted to different subcellular compartments in each fungus. In contrast, MtDef5 rapidly permeabilizes the plasma membrane of both fungi and induces accumulation of reactive oxygen species. It is also internalized by these fungi, but uses spatially distinct modes of entry into these fungi. It co-localizes with cellular membranes, travels to nucleus and becomes dispersed in other subcellular locations[2]. MtDef4 binds to plasma membrane resident bioactive phospholipid phosphatidic acid (PA), whereas MtDef5 binds to several phospholipids but with strong preference for phosphatidylinositol monophosphates, PI3P, PI4P and PI5P. MtDef5 forms oligomers in presence of PIP, PI and PA[1,2]. Thus, MtDef4 and MtDef5 exhibit different modes of antifungal action and have strong potential for use as unique antifungal agents in transgenic crops.
Aflatoxins, secondary metabolites produced by Aspergillus flavus, are extremely toxic carcinogenic compounds. Aflatoxin contamination caused by A. flavus infection of peanuts poses a major threat to public health in developing countries of sub-Saharan Africa and Asia. Transgenic peanut lines overexpressing apoplast-targeted MtDef4 have been generated. When challenged with A. flavus, peanut seeds expressing this defensin exhibit strong resistance to this pathogen and accumulate extremely low levels of aflatoxins[3]. This is the first study to demonstrate highly effective biotechnological strategy for successfully generating transgenic peanuts that are near-immune to aflatoxin contamination, offering a panacea for food safety for people in developing countries.

References

1. El-Mounadi K et al. (2016). Molecular Microbiology 100, 542.
2. Islam, K et al. (2017) Nature Scientific Reports DOI:10.1038/s41598-017-16508-w.
3. Sharma, K et al. (2017) Plant Biotechnology Journal, DOI.org/10.1111/pbi.12846