

ICRISAT researchers help raise groundnuts free of toxin

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Dual strategy involves inserting alfalfa genes into the plants to boost immunity and a gene silencing technique to prevent aflatoxin production

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Researchers at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in Hyderabad have developed dual strategies to keep groundnuts almost free of aflatoxin – a toxin produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* – contamination. While one strategy prevents groundnuts from being infected by the fungus thereby preventing the toxins from being produced, the other strategy

prevents the fungus from producing the toxin even if groundnuts somehow get infected with the fungus.

Genetic engineering approaches were used for inserting two alfalfa genes into groundnut plants to enhance immunity against fungal infection and growth. Preventing aflatoxin production even in case of any infection was achieved through a plant-induced gene silencing technique.

While both strategies showed promising results, the ultimate goal is to com-

bine the two traits into a single variety to offer double protection so that groundnuts do not accumulate any aflatoxin or the amount of toxin is well within permissible limits at or after harvest.

Combining two traits

“It is a proof-of-concept study. We have individually tested each of the two mechanisms and it is a matter of using conventional plant breeding approaches to develop a variety that has both the traits in place,” says Kiran K. Sharma from

ICRISAT. The researchers plan to start field trials early next year.

“It will take one-two years to breed the two traits into a single variety and another about three years to conduct biosafety trials followed by the development of regionally adapted groundnut varieties. So, if everything goes to plan and gets approved by the *Genetic Engineering Appraisal Committee (GEAC)*, farmers will have a groundnut variety that is near-immune to aflatoxin contamination in five to seven years,”

says Dr. Pooja Bhatnagar-Mathur from ICRISAT who led the team.

“We selected two specific genes from alfalfa and inserted them into groundnut plants to enhance the immunity against fungal infection and growth. Groundnuts showed very little fungal infection and negligible aflatoxin contamination,” says Dr. Bhatnagar-Mathur. “We choose alfalfa as it is a legume like groundnut.”

To further prevent toxin production even when groundnuts get infected with

the fungus, the researchers designed two small RNA molecules that silence the fungal genes which produce aflatoxin.

“When the fungus and plant come in contact with each other the small RNA molecules from the plant enter the fungus and prevent it from producing aflatoxin,” says Dr. Sharma, who is the first author of the paper published in *Plant Biotechnology Journal*.

About 40 hours after infection with *Aspergillus*, six lines with alfalfa genes

showed less than 1 part per billion (ppb) of toxin and another five lines showed 1-4 ppb compared with over 3,000 ppb in groundnuts that did not have these genes.

Similarly, six lines carrying the RNA molecules, the toxin present was less than 1 ppb and two other lines showed 1-4 ppb of toxin. “It is much lower than the Indian and U.S. safety limit of 20 ppb and meets even the stringent European safety limit of 4 ppb,” Dr. Bhatnagar-Mathur says.