Biotechnology and nematodes

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Banana and plantain (*Musa* spp.) are major staple foods and a source of income for millions in tropical and subtropical regions. Most of the banana grown worldwide are produced by small-scale farmers for home consumption or sale in local and regional markets.

Many pests and diseases significantly affect banana cultivation. Nematodes pose severe production constraints, with losses estimated at about 20% worldwide. Locally, however, losses of 40% or more occur frequently, particularly in areas prone to tropical storms that topple the plants.

Pest management in banana is based on several principles, primarily through the use of clean, healthy planting material, crop rotation, and in commercial settings, chemical control. However, crop rotation is often impractical, especially for small-scale farmers, while nematicides are locally unavailable or not affordable for subsistence farmers. These pesticides are also highly toxic, environmentally unacceptable, and increasingly being withdrawn from use.

Limited sources of nematode resistance and tolerance are present in the banana gene pool. Some resistance has been identified against one of the most damaging nematode species, the burrowing nematode (*Radopholus similis*), but this needs to be combined with consumer-acceptable traits. Furthermore, several species of nematodes are often present together, requiring a broad spectrum resistance able to control not just *Radopholus* but other damaging nematodes, such as species of *Pratylenchus*, *Meloidogyne*, and *Helicotylenchus*.

Enter biotechnology. Biotechnology offers sustainable solutions to the problem of controlling plant parasitic nematodes. Several approaches are possible for developing transgenic plants with improved resistance; these include strategies against invasion and migration and against nematode feeding and development.

Some successes in genetic engineering of banana have been achieved, enabling the transfer of foreign genes into the plant cells. An efficient transformation protocol for African banana cultivars has been established at IITA using meristematic tissues. The protocol avoids the callus and cell suspension culture requirements of other approaches. It is rapid, genotype independent, and avoids the somaclonal variation that often results from regenerating embryogenic cell suspensions.

IITA, in partnership with the University of Leeds, UK, is exploring the potential of biotechnology to develop plantain resistant to nematodes with funds from the Department for International Development/Biotechnology and Biological Sciences Research Council.

Prof. Howard Atkinson's group in Leeds has demonstrated that more than one independent basis for transgenic resistance provides an additive effect for nematode control. Our use of three independent additive approaches is designed to ensure a resistance level that prevents the buildup of damaging populations, even if virulent individuals completely challenge one line of defense or partially compromise them all. We intend to demonstrate that this additive approach can provide durable resistance.

The three approaches are a cysteine proteinase inhibitor (a cystatin), a potato tuber serine/aspartic proteinase inhibitor, and a repellent peptide. Cysteine proteinases are used by a wide range of plant parasitic nematodes to digest dietary protein. The cystatin prevents this digestion and slows nematode growth. Transgenic expression of both proteinase inhibitors provides effective control of both cyst and root-knot nematodes and cystatin has also been shown to be effective against *Radopholus*.

Cysteine proteinases are not present in mammals and those we will use lack toxicity or allergenicity for humans. They occur in common foods, such as the seeds of maize, rice, and cowpea, and people rapidly digest them. The other, very distinct, novel approach is the use of a repellent. This is also not lethal to nematodes or other organisms. Nematodes do not invade roots applied with repellent because they fail to detect the host's presence. This approach is effective against a wide range of nematode species.

We will also be using a novel RNA interference (RNAi) approach. The use of RNAi for functional analysis of plant

Leena Tripathi inspecting a diseased banana leaf. Photo by J. Oliver, IITA

parasitic nematode genes was first established in the University of Leeds. The approach relies on the production of double-stranded RNA molecules by banana cells. When they are ingested by the nematode, they specifically interfere with the expression of the essential nematode gene they target. The advantage of the RNAi approach is that no novel protein production is required to achieve resistance to nematodes. This offers a considerable biosafety advantage, given that RNA molecules represent no food risk and there is little likelihood of nontarget effects. The challenge is to provide an effective level of resistance to all banana nematodes by this approach. Genetic transformation of plantain using these approaches is in progress at IITA.

Gene flow is not an issue for this crop, making the transgenic approach even more attractive. Banana and plantain lack cross-fertile wild relatives in many production areas. Most edible banana are male- and female-sterile and depend on vegetative propagation. The new defense will be integrated with other pest management strategies already developed at IITA to maximize resistance levels and safeguard durability. This work is part of a new, interdisciplinary research partnership between IITA and the University of Leeds, directed at enhancing human health and food security in sub-Saharan Africa.

We also plan to stack genes for *Xanthomonas* wilt and nematode resistance into one line to produce a high-value product for farmers. Gene stacking is becoming common, adding multiple traits at once into the plant genome. Resistance to diseases and pests can be achieved by integrating several genes with different targets or modes of action into the plant genomes. We already have promising results with genes for resistance to banana *Xanthomonas* wilt (BXW) (R4D Review Edition 1), which we would like to combine with nematode resistance. Banana cultivars with resistance to multiple diseases and pests will be a breakthrough in banana improvement.