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DNA barcodes for pathogens of African food crops

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Diagnostic tools play an important role in the accurate and timely identification of the pathogens involved in disease etiology, also in disease surveillance, the development of host plant resistance, quarantine monitoring, and support safe conservation and the exchange of germplasm. Detailed knowledge of pathogen population structure and genetic diversity is a prerequisite to developing unambiguous diagnostic tools and is critical in establishing disease management tactics. Increasingly, modern diagnostic tools are being based on the DNA characteristics of the pathogen as they are neutral to growth stage and environment; offer adequate diversity to distinguish species, strains, substrains, isolates, and even individuals; and offer convenience of detection using modern bio-techniques such as polymerase chain reaction (PCR).

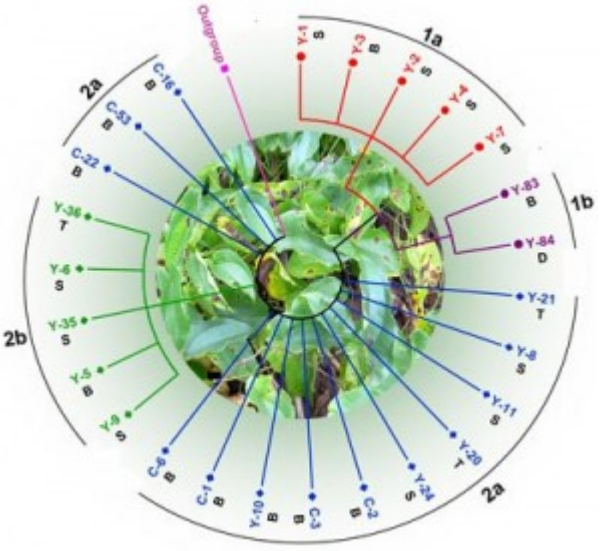
Severe anthracnose symptoms on cassava stem. Photo by R. Bandyophadyay, IITA.

At IITA, we undertook a new initiative to characterize pathogen populations and recognize unique stretches of sequences—known as ”DNA barcodes”—that can be used as genetic markers for the rapid diagnosis of the pathogens and pests affecting the African food crops on which we work. DNA barcodes, otherwise also known as DNA markers or DNA fingerprints, are essentially a short stretch of nucleotide sequences that aid in the specific identification of species strains or substrains. They are used to resolve pathogen taxonomy and phylogeny.

The work focuses on economically important fungal, viral, and bacterial pathogens, insects, and nematodes. The information is used to gain ”barcode”

designation in global sequence databases such as BOLD (the barcode of life data system) or NCBI (National Center for Biotechnology Initiative), and to assemble these into a database for public access.

This approach—a combination of conventional biology, biotechnology, and bioinformatics—involves the selection of targets, amplification of target genes using universal or generic primers, sequencing of target genes and identification of unique barcodes, and development of PCR-based diagnostics for specific detection of barcodes. This approach is particularly useful in identifying pathogens that are difficult to distinguish either by morphology or other properties. It offers high accuracy in identifying quarantine pathogens and reduces the risk of spread. In addition to diagnosis, it also contributes to the fundamental understanding of pathogen phylogeography and relationship with host and contributes to the development of management tactics.



Clustering of 25 yam isolates based on rDNA sequences. Courtesy of Lava Kumar, IITA.

We are using this approach to characterize the fungal pathogen(s) causing anthracnose—the most destructive disease of yam and cassava in West Africa. The disease causes severe yield losses in both crops and often kills the plant. The causal fungus, *Colletotrichum gloeosporioides* Penz., is widespread in West Africa. We identified various isolates of this fungus differing in morphology, growth characters, and pathogenicity, then investigated their genetic relatedness and diversity through molecular analysis of a set of 25 reference isolates (17 from yam and 8 from cassava) using multilocus gene targets. They were grouped into spot (S) and blight (B) isolates based on symptoms they induce. Both types of isolates infect yam, but only B isolates infect cassava. We assessed the genetic diversity in these isolates by nucleotide sequencing and cluster analysis of the ~540 base pair (bp) nuclear ribosomal internal transcribed spacer region (ITS1, ITS2 and the 5.8S gene) and partial gene sequences of actin (~240 bp) and histone (~370 bp).

Phylogenetic cluster analysis grouped the 25 isolates into two major clades (a clade is a group that shares features from a common ancestor) and two

subclades within the major clades. Both the S and B isolates were distributed between the two clades (see figure). All the isolates in clade 1 were unique to yam. Seven of these isolates (YA08-1, YA08-2, YA08-3, YA08-4, YA08-7, Y-83, Y-84) formed a genetically distinct lineage, indicating that they could be new strains unique to yam. Isolates in clade 2 infect both cassava and yam, suggesting their capability to infect a wide range of plants. It is plausible that clade 2 isolates could be those most frequently occurring on yam and cassava because of their ability to survive on weeds and other crops. We recognized unique sequence motifs and designed diagnostic PCR primers directly from infected plant tissues for the specific amplification of *C. gloeosporioides* infecting yam and cassava.

Using a similar approach, we characterized the fungal agent associated with gray leaf spot (GLS), the most destructive disease of maize. We found that GLS in Nigeria is caused by a distinct species of *Cercospora*, but not *C. zeae-maydis*, a previous conclusion derived from conventional analysis. This work, in addition to confirming the GLS etiology, allowed us to establish a unique set of primers for the specific identification of the GLS pathogen prevalent in Nigeria.

Through comparative genomics, we identified common genome regions in cassava mosaic begomoviruses occurring in sub-Saharan Africa. We developed a simple multiplex PCR assay that can detect all the major viruses in cassava mosaic disease etiology. This test has been adopted for virus indexing of cassava propagated in vitro.

To aid us in diagnostics research, we developed a simple and cost-effective procedure suitable for extraction of DNA from seeds, leaves, stems, tubers, and even roots. The resultant DNA is suitable for PCR-based diagnoses of fungi, bacteria, and viruses in the infected tissues of a wide range of plant species. It is handy for the quarantine monitoring of germplasm. We are establishing a repository of diagnostic protocols in an approach we call the “Diagnostic Basket®” and will make it available to users.

Barcodes and diagnostic tools provide a solid base for the understanding of the taxonomy and diversity of pathogens infecting African food crops.