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Cover: Research worker in IITA's maize field collecting tassels for hand pollination.
Photo by C. Ono-Raphael.

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Molecular diagnostic tools for plant health protection

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Molecular tools in disease diagnosis

Rapid advancements in biotechnologies have led to the development of a myriad of molecular diagnostic tools in the past decade[1]. These tools, either based on the properties of nucleic acid (DNA or RNA) or proteins of the target agents, have improved the efficacy, accuracy, and speed of detection and identification of disease-causing agents and characterization of the diversity of pathogens and pests.

Most popular protein detection methods depend on antigen-antibody interactions. Polyclonal or monoclonal antibodies produced against the proteins of interest are used as probes to detect the target proteins by techniques such as enzyme-linked immunosorbent assay (ELISA), Western immunoblotting, dot immunobinding assay, and several variants of these techniques. Meanwhile, nucleic acid-based diagnostic tools are based on the hybridization of homologous nucleotides, size of the DNA fragments generated by restriction enzyme treatment, order of nucleotide arrangement, or a combination of more than one of these approaches. Polymerase chain reaction (PCR), developed in the mid-1980s, has led to the development of several new and simplified techniques, fast established as a mainstay of applied molecular biology and molecular diagnostics.

Researcher observing mouse hybridoma cell lines under microscope in the Virology and Molecular Diagnostics Unit, IITA, Ibadan, Nigeria. Photo by IITA.

L. Kumar, Head of Germplasm Health Unit and Virologist, IITA, Ibadan, Nigeria.

Platform for developing molecular diagnostics

The objective of the molecular diagnostics research in IITA is to develop tools and technologies for better understanding, diagnosis, and monitoring of biological systems. This program emphasizes the development of simple and accurate tools and procedures for rapid identification of pathogens and pests affecting the food and horticultural crops in sub-Saharan Africa (SSA). Both protein and nucleic-acid based diagnostic tools have been developed against target agents (viruses, fungi, bacteria, phytoplasma, insect pests, and mycotoxins). These tools are critical to several programs on crop improvement and crop protection, including evaluation of germplasm for host resistance, breeding for pest and disease resistance, surveillance surveys, and monitoring programs.

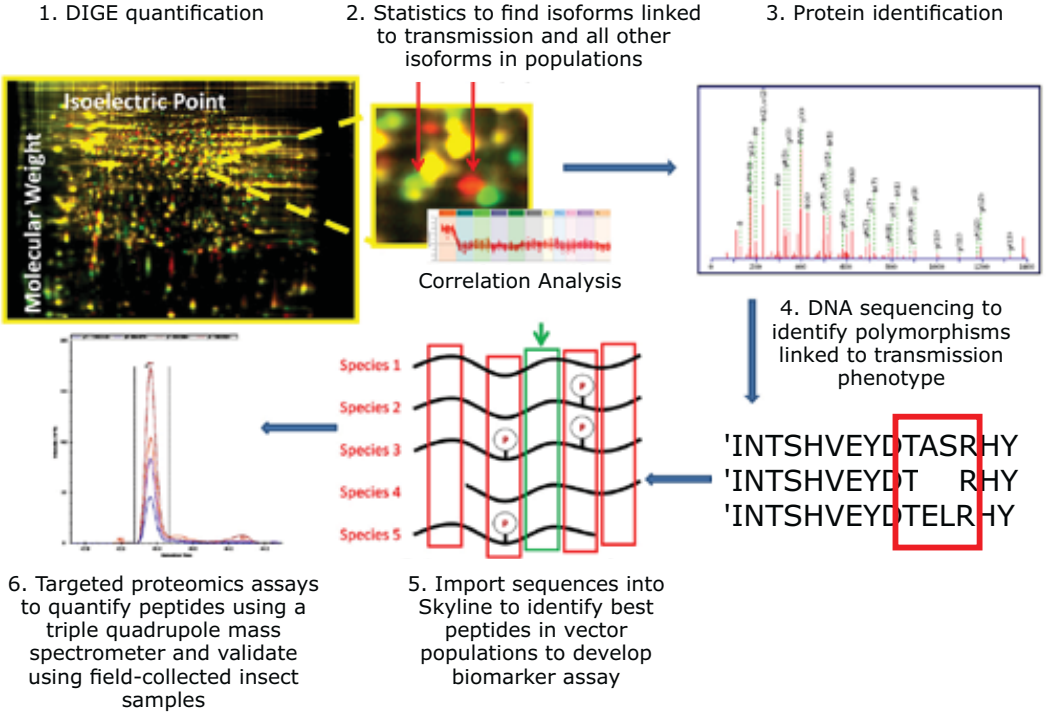
ELISA-based diagnostics are preferred for the identification of plant viruses. It is simple, reliable, cost-effective, and easy to adopt in minimally-equipped labs. Backed with facilities for purifying proteins, and production of polyclonal and monoclonal antibodies, ELISA-based diagnostics were established for about 20 economically important viruses affecting IITA's mandate crops in SSA (e.g., Maize streak virus, cassava mosaic begomoviruses, Cowpea mottle virus, Southern bean mosaic virus, and more). Antibodies were also produced against nonviral targets such as mycotoxins. Polyclonal antibodies produced against aflatoxin B1 were used to develop the 'Afla-ELISA' test for quantitative estimation of aflatoxins in maize and other commodities (see article on page 45). Monoclonal antibodies are usually produced for discriminating closely related virus species or strains (e.g., African cassava mosaic virus and East African cassava mosaic virus). The production of monoclonal antibodies is expensive and tedious, but it offers the advantage of perpetual production of antibodies from mouse hybridoma cell lines. Because of this, IITA has placed increasing emphasis on producing monoclonals for all important pathogens.

PCR-based diagnostics are developed as an alternative tool or to overcome the limitations of ELISA in detecting viroids, viral satellites, and to discriminate strains and closely related species. Oligonucleotide primers have been developed based on the genomic data generated from our research programs and those available in the public database for the specific detection of targets in PCR assays. Procedures were also established to simplify PCR application. For instance, a procedure established for direct detection of viruses in leaf sap bypasses the need for nucleic extraction[2]. Emphasis is placed on the development of multiplex PCR assays for the simultaneous detection of more than one virus in a single reaction. A multiplex PCR method has been developed for the simultaneous detection of African cassava mosaic virus and East African cassava mosaic like-viruses responsible for cassava mosaic disease in SSA[2]. This test was further improved to detect cassava brown streak viruses that have emerged as a major threat to cassava in East Africa, thereby making it a one-stop test for detecting all the major viruses infecting cassava in SSA.

Similar efforts are being devised to detect all viruses infecting yam. Real-time PCR using TaqmanTM probes are being developed to quantify virus concentrations within the plants to characterize host response to virus inoculation. Presently, specific and generic diagnostic tools for the detection of almost all the pathogens that affect major food staples in SSA have been established at IITA.

Pathogen diversity and DNA barcodes

Detailed knowledge of pathogen diversity is a prerequisite to developing unambiguous diagnostic tools. Pathogen populations are characterized by sequencing the specific genes and the data generated is used to interpret origin and spread of the pathogen, taxonomy, and phylogeny. For diversity assessment, gene targets are selected based on the pathogen that comprise, ribosomal Internal Transcribed Sequence (ITS), mitochondrial cytochrome oxidase-I (COI), histone, virus coat protein, etc. This approach has been used for assessing the diversity of *Colletotrichum gloeosporioides* responsible for anthracnose of yam, *Cercospora* spp. causing gray leaf spot of maize, cassava brown streak virus, banana bunchy top virus, and several other agents. Information generated from these studies have provided valuable clues to understand the origin and drivers of spread, identification of previously uncharacterized pathogens[3,4] and identification of unique markers known as "DNA barcodes" for use as genetic markers for identifying pathogens and pests[5].



Workflow in development of protein biomarkers. Source M. Cilia, Cornell University

Biomarkers for insect vectors

Recently a new initiative was started in collaboration with Cornell University to identify protein biomarkers to rapidly identify variation in vectoring potential of aphid and whitefly vector populations. Diagnostic tools developed in this program will aid in better understanding the virus-vector interactions, disease epidemiology, and improved management of insect vector-borne virus diseases.

Training in application of molecular diagnostics

In addition to technology development, efforts are made to transfer technology, products, and skills to stakeholders in national research and extension services. This is done through collaborative activities and organization of training courses at regular intervals in collaboration with national organizations such as the Nigerian Institute of Science Laboratory Technology. During the training courses, specific emphasis is placed on the application of diagnostics in monitoring and surveillance programs. Standard diagnostic protocols are compiled into a cook-book style laboratory manual[6] and distributed during the training courses.

End note

Molecular diagnostics development programs in IITA consider the latest knowledge and state-of-the-art technologies in establishing simple and robust tools that are relevant to end-users, are low-cost, and conducive for adoption in minimally equipped labs. We are adding new tools, such as, loop-mediated isothermal amplification reaction (LAMP) assay and deep sequencing approaches to broaden the knowledge on pathogens occurring in our mandate crops to increase the repertoire of available tools.

Molecular diagnostic tools are routinely used in germplasm indexing, phenotypic evaluation of germplasm, disease surveillance, and monitoring programs in SSA. They are also used in collecting baseline information and monitoring shifts in pathogen and pest dynamics due to changes in agricultural systems and climate change effect. These tools are already proving useful in rapid detection and identification of new and emerging pathogens and pests [e.g., *Paracoccus marginatus* (papaya mealybug) in Nigeria; *Phytophthora colocasiae* causing taro leaf blight in Nigeria and Ghana; 16srII group phytoplasma responsible for witches' broom disease of soybean in Southern Africa; and Banana bunchy top virus in Benin].

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