R4D Review

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Multiplying cassava somatic embryos under sterile in vitro conditions.

Photo by J.Oliver, IITA

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PUBLISHER'S NOTE

Is biotechnology a panacea?

Biotechnology is often understood to mean a single technology, In reality it is a collection of technologies that can be applied to address many challenges in agriculture (crop and animal health, food production) pharmaceuticals, and medicine. Biotechnology is often seen as a panacea which is not the case; it is one more tool, albeit an important one, in the arsenal of tools used against the challenges humanity faces. In agriculture, the technology can help accelerate the development of crops resistant to insects and disease, the development of new uses for agricultural products, livestock vaccines, and improved food qualities. African institutions from Cairo to Cape Town, from Dakar to Dar-es-Salaam are using biotechnology in diverse ways.

IITA's position on biotechnologies is similar to that on all other sciences. We think Africa, its ministries, universities, teaching hospitals, and other research institutions, should not be excluded from any science. Just the need to know, so as to advice governments on the usefulness of a technology to a country's needs, requires their involvement and knowledge in that science. Whether a particular product of that technology, e.g., genetically modified crops, is adopted or not, is a decision made by governments and not by scientists.

Although many African governments are on the brink of embracing the promised benefits of biotechnology, they have not totally committed in terms of providing government funding for more research in agricultural and social/economic development, or policy support for science. What is needed is for R4D institutions, such as IITA and its partners to continue to provide knowledge about these important technologies and their possible impact on sub-Saharan Africa.

This issue highlights some of the cutting-edge work that IITA and its partners (AATF, NARS, donors, NGOs) are doing to help find solutions to problems in tropical agriculture, and thus provide more food and improved livelihoods for the millions of people depending on agriculture. The R4D Review welcomes feedback and comment about any of the information and work featured in this issue. We encourage you to visit the online R4D Review at www.r4dreview.org.

"Whether a particular product of that technology, e.g., GMOs, is adopted or not, is a decision made by governments, not scientists."

—Hartmann, IITA Director General

A vibrant local market in Ibadan, Nigeria. Photo by IITA

NEWS

East and Southern Africa hub

IITA launched its new East and Southern Africa (ESA) hub this year to give priority attention to research in banana, plantain, and cassava, and to provide better services to scientists in those two regions. Based in Dar-es-Salaam, Tanzania, it will also carry out research on other IITA mandate crops, such as yam, maize, and soybean.

The hub, according to IITA Director Steffen Abele, will look at the diversification of banana breeding programs from the more highland-oriented sites to coastal regions, where there is a huge diversity of banana that can be exploited. Attention on banana and cassava is expected to go a long way in improving the livelihoods of resource-poor farmers and in alleviating poverty.

The ESA hub focuses on Eritrea, Ethiopia, Somalia, The Sudan, Uganda, Rwanda, Burundi, Kenya, Tanzania, DR Congo, Malawi, Zambia, Mozambique, Zimbabwe, Swaziland, Lesotho, Angola, and Botswana.

2008 science awards

IITA won two major CGIAR Science Awards for innovations in developing and promoting postharvest technologies and agricultural communications strategies to reduce hunger and poverty in sub-Saharan Africa. The awards are for Outstanding Agricultural Technology in sub-Saharan Africa and Outstanding Communications. They were announced during the CGIAR's 2008 Annual General Meeting held in Maputo, Mozambique,1–5 December 2008.

Dr Lateef Sanni Oladimeji, of the University of Agriculture in Abeokuta, Nigeria, IITA's Postharvest Specialist, received the 2008 CGIAR Regional Award for Outstanding Agricultural Technology in sub-Saharan Africa.

His expertise on drying technologies has contributed to considerable gains in income and employment for numerous small- and medium-scale enterprises in Nigeria and several other West African countries.

Sanni initially designed a rotary dryer that increased production of cassava flour to 300 kg every 8 hours. This was disseminated to cassava processing facilities in southwest and southeast Nigeria. More recently, within IITA's Integrated Cassava Project, he assembled a team of engineers who designed a "flash" dryer capable of drying 250 kg/hour of cassava flour. His work has helped to increase the use of locally-manufactured flash dryers in Nigeria from two units before 2003 to over 60 units today.

Dr Soniia David, IITA Regional Participatory Extension Specialist, and her team at the Sustainable Tree Crops Program, received the 2008 CGIAR Science Awards — Outstanding Communications Category — for training farmers in West African countries to use digital video cameras as a way to share knowledge on sustainable cocoa production (see related story on pages 24–25). By setting up video viewing clubs (VVC), the team got together groups of farmers to watch and learn from the videos.

Video viewing club, Ghana. Photo by S. David, IITA

Flash dryer, IITA. Photo by O. Adebayo, IITA

To date, 450 farmers in Ghana have participated in VVCs. Farmers who adopted the crop and pest management practices promoted by the YouTube videos are likely to increase yields by 20–40% and decrease pesticide use by 10–20%.

USAID project

A USAID-funded project seeks to further increase cassava production in farmers' fields by 30% in seven sub-Saharan African countries. The project is called "Unleashing the Power of Cassava in response to the food price crisis (UPoCA)."

It aims to maximize the use of cassava to address food price crises in Nigeria, DR Congo, Ghana, Malawi, Mozambique, Sierra Leone, and Tanzania. IITA and its national partners across the seven countries will implement the US$5.3 million project.

Dr Braima James, Project Manager, said that the primary focus of the project was to expand and sustain on-farm productivity and the profitability of cassava. “This will be achieved through the distribution of elite varieties and related inputs; dissemination of appropriate integrated crop management techniques to ensure at least 30% increase in root yields; and the promotion of entrepreneurship in cassava planting material supply.

"About 267,000 farmers are expected to benefit from the project," Dr Richardson Okechukwu, Assistant Project Manager, says. "The beneficiary-farmers are currently harvesting 7–12 t/ha across the seven countries but the project aims to raise yields to 12–30 t/ha."

Yam-growing technique

IITA and partners have developed a tuber-less yam propagation technique. In this innovative approach, yam is propagated through vine cuttings, using carbonized rice husks as the growth medium. The method signficantly lowers the risk of nematode infestation from using tubers as planting material, promotes faster multiplication, better and more uniform crop quality, and makes more yam available for food or for sale.

In the traditional system, tubers used as seed take up 30–50% of the production cost. The system is also quite inefficient: the resulting multiplication rate is only about 1:5–10. In comparison, cereals, for instance, have a propagation ratio of about 1:300.

The technology offers a rapid, clean, and cost-effective mass method of multiplying yam. It could effectively address the need for fast and wide distribution of high-quality improved varieties to meet the increasing demand for the crop.

The research is funded by the Japanese government, the Sasakawa Africa Association, Tokyo University of Agriculture, and the International Cooperation Center for Agricultural Education, Nagoya University, Japan. Partners include the Tokyo University of Agriculture, National Root Crops Research Institute in Umudike, Nigeria, Crop Research Institute in Ghana, and the Institute of Agricultural Research for Development in Cameroon.

Yam seedlings.
Photo by O. Adebayo, IITA

Growing cassava
in freezing cold Denmark

Kirsten Jørgensen and Birger Lindberg Møller

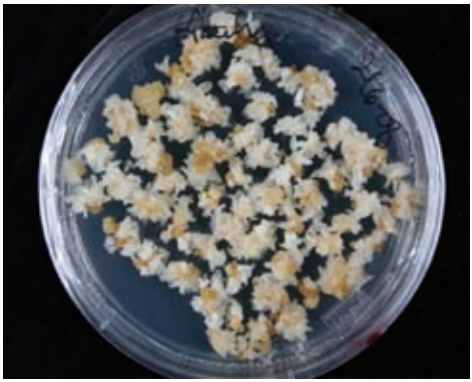
Cassava, that tropical tuber that is a staple to millions in sub-Saharan Africa, is being grown in freezing Denmark—at the University of Copenhagen. The precious plants are grown in the greenhouse during the dark winter days when the snow is lying on the roof.

The crop has been a prime focus of the University's research because of its importance as a food security crop and commodity in economic development. It has a high content of cyanogenic glucosides, toxic substances which may constitute a nutritional problem in regions where cassava is the dominant or sole staple food. During processing, cyanogenic glucosides are converted into cyanohydrins, ketones, and hydrogen cyanide. These are all toxic and should not be consumed in excessive amounts.

Cyanogenic glucosides are an ancient group of bioactive natural products present in crop plants, forage plants, and important trees. More than 3,000 plant species are cyanogenic, including cassava, apricot, cherry, clover, flax, barley, sorghum, wheat, bamboo, eucalypt, and poplar. We study most of these plant species to understand these compounds in terms of their synthesis, turnover and regulation, and their biological function. In these studies, we use model plants such as *Lotus japonicus*. *Lotus* contains linamarin and lotaustralin, the very same cyanogenic glucosides found in cassava. The genome sequence of *Lotus* has been sequenced, and the use of transgenic model plants is a key tool in our studies.

Our laboratory was a world-first in isolating all three genes for cyanogenic glucoside synthesis. This work was done in sorghum. We were also a world-first in isolating the genes responsible for cyanogenic glucosides synthesis in cassava.

Our research aims to control the level of cyanogenic glucosides in different parts of the cassava plant. It involves understanding the regulation of the biosynthetic genes and the turnover and transport processes. We use tools such as the relevant omics platforms for these studies, including metabolomics where we use high-pressure liquid chromatography and mass spectrometry to determine the constituents found in the different cassava tissues as a result



Embryo culture of Kibaha. Photo by K. Jørgensen

Associate Professor, VKR Research Centre "Pro-Active Plants", Plant Biochemistry Laboratory, Department of Plant Biology and Biotechnology, University of Copenhagen, and Professor, Plant Biochemistry, The Royal Veterinary and Agricultural University and Faculty of Life Sciences (LIFE), University of Copenhagen, Denmark; also Director, VKR Research Centre "Pro-Active Plants"; Deputy-Director, UNIK Synthetic Biology; and member, IITA Board of Trustees.

of environmental challenges. A second important tool is transcriptomics. In collaboration with IITA researchers, such as Dr Ivan Ingelbrecht, we have designed a cassava DNA chip (see related story on pages 26–29) that allows us to monitor the profile of gene expression and how these profile changes for individual genes respond to plant development, nutritional status, and environmental challenges.

In the long term, we want to produce a virtual model of the cassava plant that would enable us to predict responses during growth and development, and to environmental stimuli. In these studies, changes in cyanogenic glucoside content, in the levels of the enzymes and genes controlling their synthesis, breakdown, and transport are given special attention.

Our studies will provide information on the level of natural variation from one plant to another in terms of interesting characteristics. To facilitate identification of individual plants with interesting properties, high throughput screening technologies have also been implemented. Cassava is a tetraalloploid plant. This makes traditional breeding very time consuming and complicated. The development of transgenic approaches where multiple gene copies may be “knocked out” in a single step offers great opportunities to develop varieties with an optimal content and distribution of cyanogenic glucosides.

Progress in cassava research is slow, because few research groups in the world are working on cassava. Typically, the tool boxes successfully used in wheat, maize, and rice breeding are not available in cassava. This makes breeding of new varieties cumbersome and time-consuming because many of the techniques have to be set up from scratch. One such example is the development of a transformation protocol for cassava that would enable us to knock out or insert new genes in elite cassava cultivars.

We have devoted a lot of effort to develop such a transformation system. This includes tissue culture work to establish protocols enabling us to produce embryogenic cultures and to regenerate plantlets from these. Likewise, reliable procedures for *Agrobacterium*-mediated gene transfer for elite cultivars had to be optimized. In this research we took advantage of the pioneering work on cassava transformation carried out at the Swiss Federal Institute of Technology (ETH) in Zürich. The system we now use is robust and we are able to transform elite lines from IITA that are high yielding and that have optimal resistance to disease and pest attack. Our transformation technology has been transferred to IITA. In 2007, we also transferred the technology to obtain cassava transformants to the Danforth Plant Science Centre, USA.

Transgenic shoots from TME12.
Photo by K. Jørgensen

It is important to select the optimal cassava lines for our research. We want to produce cassava lines appropriate for end-users. There is not much value in engineering interesting agronomical traits into lines that have no value to the end users, i.e., the farmers and consumers. This is yet another example where close collaboration with IITA researchers has greatly benefited us. Collaboration with IITA helped us to focus our work on agriculturally important lines.

In the initial phase of our work to reduce cyanogenic glucoside synthesis, we used constitutive promoters such as that from the 35S cauliflower mosaic virus. We have now shifted our focus to using native cassava promoters that

more efficiently target the cells in the tubers where, for example, synthesis of cyanogenic glucosides takes place, thus providing better control. The content of cyanogenic glucosides varies among vegetatively propagated and thus genetically identical plantlets. Accordingly, multiple tests at different growth stages have to be carried out to determine the degree of downregulation (reduction) of cyanogenic glucoside synthesis. This makes the procedure to find the right lines time-consuming.

The protein content in current elite cassava cultivars is very low partly perhaps because of the continued breeding for high starch yield. In the past, breeding for high protein content would be at least partly in vain because a significant proportion of the protein is lost anyway during processing when the cyanogenic glucosides and their toxic degradation products are removed to provide food safe for consumption.

Using molecular breeding, there are several ways to achieve transgenic lines with enhanced protein levels in the tubers. One way is to identify specific storage proteins from wild cassava varieties that exhibit a high content of essential amino acids, such as methionine and lysine, and then express these nutritionally beneficial proteins in the elite lines. Another way would be to use storage proteins from other known species, such as patatin from potato. We are currently transforming African elite lines with constructs encoding patatin that will be incorporated into the starch grains in the cassava tuber.

The original focus on the molecular breeding of elite cassava cultivars with a controlled and reduced content of cyanogenic glucosides and a higher protein content has recently been expanded to incorporate varieties carrying yellow tubers. These have increased levels of carotenoids that are precursors of vitamin A. IITA provided the cassava lines with high carotenoid content obtained by classical breeding.

Transgenic acyanogenic cassava (TME12) in greenhouse. Photo by K. Jørgensen

The long-term aim of our research is to improve the nutritional value of cassava tubers from African elite cultivars by blocking the accumulation of cyanogenic glucosides, enhancing the protein content, and increasing the pro-vitamin A content. Future goals involve engineering resistance to important pests and diseases in the very same lines.

Climate change has spurred a worldwide demand for drought-tolerant plants, such as cassava. Likewise, as Western industrialized countries move towards a bio-based society less dependent on fossil fuels, starchy plants that can produce very high yields under optimal growth conditions become key targets for research. As part of these efforts, the cassava genome is now being sequenced in the US. When the genome sequence is available research on cassava will be that easier and may be the first step in developing the crop as an efficient environmentally benign “green factory” for producing valuable chemicals and pharmaceuticals.

The cassava group at the University of Copenhagen is headed by Professor Birger Lindberg Møller and principal investigator Associate Professor Kirsten Jørgensen, together with Assistant Professor Rubini Kannangara, Technicians Charlotte Sørensen, Evy Olsen, and Susanne Bidstrup, and gardener Steen Malmmose.

http://www.proactiveplants.life.ku.dk

Biotech in Nigeria:
The journey so far

Nigeria, the world's largest grower of cassava, producing over 40 million tons per year, is seeking to adopt the use of modern biotechnology tools in agriculture, but efforts are stymied by the absence of a biosafety law.

The passage of the bill by the Nigerian Parliament will launch the country into the production and commercialization of genetically modified organisms (GMO) with the capacity to increase crop production, ensure food security, and improve rural livelihoods.

"The passage of the bill will be great," said Dr Oyekanmi Nash, Program Director, West African Biotechnology Workshop Series. "Biotechnology holds the key to some of our problems in agriculture and health, and the earlier we tap into it, the better," he added.

Background

Currently, Nigeria's population of more than 140 million with an annual growth rate of 2.9% demands increased agricultural production to guarantee food security.

This means traditional agricultural practices, characterized by the use of poor seedlings, must give way to modern tools to allow agriculture to grow by double digits from the current average of about 6%. Such a growth will conserve government revenues from being used in importing food items.

According to government figures, the country spends about US$3 billion annually on food importation. The situation was worse in 2008 when food prices hit the roof, aggravated by the negative effects of severe drought on agricultural production in the northern parts of the country, and high energy costs when crude oil reached $150 per barrel.

"With the turn of events now and for us to meet our food demand in the future, we should apply modern biotech in crop production," Nash said.

He commended IITA for setting up a modern biotech laboratory in Nigeria, saying that the establishment of such a multimillion dollar laboratory in Nigeria was a reflection of the institute's commitment to fight poverty in Africa and improving rural livelihoods.

Challenges in introducing GMOs

If the biosafety bill is passed, Nigeria will join other African nations, such as Burkina Faso, Egypt, and South Africa in cultivating GMO crops.

It is expected that the entrance of GMOs will increase crop productivity, lower the cost of production, guarantee food security, and improve both the health and livelihoods of resource-poor farmers who make up more than 70% of the rural population.

The absence of a biosafety law is the problem. In addition, research and development in GM crops are indeed in their infancy in Nigeria as very few establishments in the national agricultural research system have developed the critical mass of human capacity and the infrastructural requirements

Nigeria's biotech milestones

- Population: about 140 million
- Annual agricultural growth rate: about 6% average
- Needs to put in place a biotech policy and set up national biotech institutions
- Signed the Cartagena protocol on biosafety in 2000
- Adopted National Biosafety Guidelines in 2001
- Drafted a biosafety bill in 2006

Fluorescence-based genotyping for DNA fingerprinting of plants and pathogens. Photo by IITA

that would lead to the accelerated development of transgenic materials.

A communiqué issued last year by stakeholders, including the National Biotechnology Development Agency (NABDA) said that other limitations in the commercialization of GMO crops included poor capital equipment, irregular energy, inadequate water supply, and ineffective use of information and communication technology, among others.

The meeting further noted the obvious deficiencies in both the teaching and learning curricula at all school levels and accordingly recommended vibrant and dynamic curricula to generate appropriate labor to meet research and development needs in biotechnology activities.

Biotech and Nigeria's vision 2020

In the next 11 years, Nigeria intends to be ranked among the top 20 economies of the world. Achieving this goal requires adopting policies and options that will lead to improved agriculture and food security among other benefits.

For Nigeria, experts say this will include genetic improvement in the priority crops such as sorghum, cassava, cotton, yam, banana, plantain, maize, wheat, gum arabic, cowpea, and soybean that are of critical importance to the nation.

Prof. Bamidele Solomon, Director-General of NABDA, which has the mandate to promote, coordinate, and regulate biotechnology across the country, said his agency would ensure that the cutting-edge technology of biotech promotes a healthy environment, ensuring national food security and providing affordable health care delivery as well as the alleviation of poverty.

While 2020 appears rather far away, not taking proactive steps toward tackling the present challenges facing the full implementation of biotech will certainly make Vision 2020 a mirage, as far as food security is concerned. This is indeed a wake-up call. The earlier we act, the better.

Tackling the banana **menace**

Lava Kumar and Rachid Hanna, l.kumar@cgiar.org and r.hanna@cgiar.org

Banana bunchy top disease (BBTD) is emerging as a serious threat to banana and plantain production in several regions in West-Central and Southern Africa. IITA is undertaking research to understand the factors leading to the increase in BBTD incidence and spread with the ultimate goal of developing integrated strategies to protect bananas from this menace.

The livelihoods of over 70 million people are intimately linked to banana (banana and plantain), a major food staple and premier fruit. It occupies an important position in the agricultural economies in sub-Saharan Africa (SSA). Banana plantations produce fruits all year round, providing farmers with food and income, even during fallow seasons, thus contributing to food security.

Various types of banana are grown in SSA: plantain in the humid lowlands of West and Central Africa; highland cooking banana in East Africa; and introduced dessert banana in all the subregions.

BBTV-infected plants in DR Congo.
Photo by L Kumar, IITA

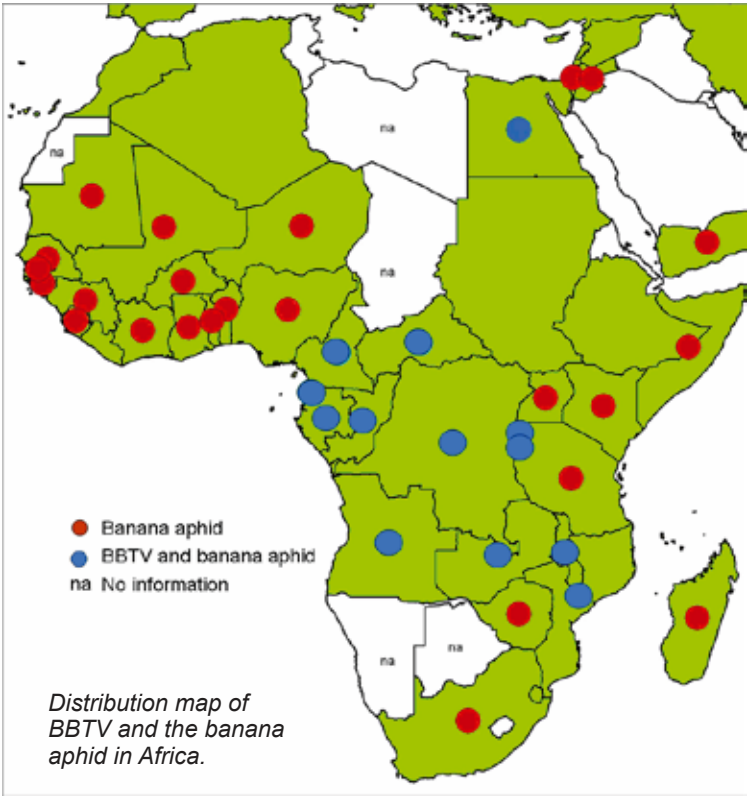
The world's greatest variability in the crops is held in West and Central Africa for plantain and Great Lakes Zone in Eastern Africa for highland banana. These regions are considered as secondary centers of banana diversity.

The foe

Bunchy top disease caused by banana bunchy top virus (BBTV) is emerging as a serious threat in several regions in Central and Southern Africa. This disease, first reported from Fiji in 1889, has been dubbed as one of the most destructive plant viral diseases. BBTV has been found in Asia, Africa, Australia, and the South Pacific islands. It belongs to the genus Babuvirus of the family Nanoviridae. The virus readily spreads in vegetative material such as corms and suckers. It also relies on the banana aphid, *Pentalonia nigronervosa*, its sole biological vector.

BBTV curtails plant growth, resulting in unproductive plants. Leaves of infected plants have pale chlorotic margins, dark green dots, and streaks along the veins which often extend down the midrib and petiole. Emerging leaves become progressively smaller and choked in the throat of the plant creating the "bunchy" appearance at the top.

Plants infected at an early age rarely produce fruit and eventually die. Those infected at a later growth stage may produce bunches, often with deformed fruits, but suckers have typical symptoms and remain unproductive. The Invasive Species Specialist Group (ISSG) of the World Conservation Union (IUCN)



Distribution map of BBTV and the banana aphid in Africa.

included BBTV in the list of 100 of the World's Worst Invasive Alien Species (www.issg.org/database).

Formidable challenges

Usually viral diseases are effectively controlled through the use of resistant sources. So far, banana varieties resistant to BBTV have not been found. Some types are tolerant or express symptoms relatively slowly. Eradication and exclusion are the only options found to be effective in controlling BBTV. These phytosanitary approaches rely heavily on early detection and destruction of the diseased mats, coupled with strict quarantine and indexing procedures to prevent further spread.

For instance, in Australia, strict legislation prevents the transfer of plant material from infected to uninfected areas. Skilled workers conduct regular inspection to identify and destroy any plant displaying disease symptoms. Thus, disease incidence has been kept at a very low level. In countries such as India and Pakistan, where effective eradication was not implemented, BBTV remains a major constraint.

Rigorous enforcement of phytosanitary procedures is expensive and extremely difficult to implement in SSA where socioeconomic conditions are poor and capacity is inadequate. In addition, banana cultivation is mainly carried out by subsistence farmers and large areas occur in the wild. Under these conditions, eradication becomes very difficult without major social upheavals. More research is necessary to devise control strategies that are congenial for application in SSA.

BBTV distribution in Africa

In Africa, the virus was first reported from Egypt over a century ago. In the 1950s, it was reported from Central Africa. The origin of this virus is not clear, but it may have been introduced into Africa through infected suckers brought by migrants from South Asia. Subsequent spread might have occurred from the movement of infected suckers by humans and natural spread by the vector aphids.

Despite its presence for at least five decades, the disease attracted very little attention until its recognition in the mid-1990s in Burundi, Malawi, and Rwanda where it was causing serious epidemics.

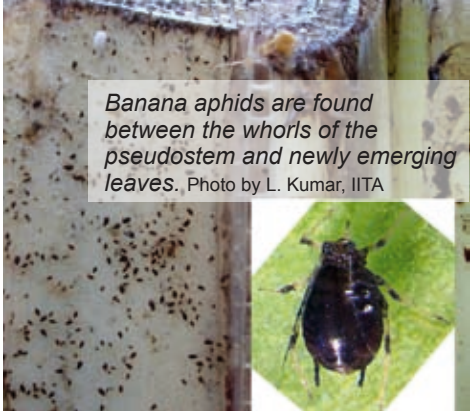
Our surveys from 180 sites in West, Central and Southern Africa point to 1994-1996 as the years when there was widespread appearance of the disease.

Presently, BBTV has been identified in Angola, Burundi, Cameroon, Congo, DR Congo, Equatorial Guinea, Gabon, Malawi, Mozambique, Rwanda, and Zambia.

Banana aphid and biocontrol

The banana aphid is widespread in SSA. Considerable variations in abundance were observed in our studies depending on locality, banana variety, and season. The

Banana with typical bunchy top disease symptoms. Photo by L. Kumar, IITA



Banana aphids are found between the whorls of the pseudostem and newly emerging leaves. Photo by L. Kumar, IITA

aphid is likely to be responsible for much of the local spread of the disease and to some extent its long distance transport. Unlike annual crops, where the fallow period acts as a check for vectors, the perennial nature of banana allows aphids to survive all year round, aiding virus spread.

Aphid control will play a significant role in BBTD management. Chemical control is expensive and not economical for farmers.

The banana aphid is exotic to Africa and lacks indigenous natural enemies. Classical biological control is being explored that includes testing known natural enemies and exploration for new ones for introduction into Africa. IITA has successfully used this approach to control several exotic pests. Biological control could minimize the local spread of the disease, and perhaps reduce it to very low levels, when coupled with tolerant varieties.

Prospects

At present, BBTV spread is not controlled in SSA. In Nkhotakhota district in Malawi, small-scale farmers have given up banana growing. Besides its effect on food security, the virus poses a serious threat to the diversity of plantain and highland banana.

Understanding its disease ecology, demographics of distribution, vector biology and ecology, awareness creation, and conventional and nonconventional approaches to tackle the virus and vector is expected to provide a reprieve in the medium term and sustainable solutions in the long term.

Is hybrid rice the answer?

Glenn B. Gregorio, IRRI (g.gregorio@cgiar.org)

The recent scarcity of rice and steep rise in price underline the need to accelerate the introduction and adoption of higher yielding varieties. This starts from having good seeds or varieties. Once a good variety is born, technology and business can work together to raise production.

The appetite for rice has continuously increased in Africa, with imports accounting for almost one-third of the total world trade in rice. Imports have increased over time as rice production grows far more slowly than total demand. It is expected that demand from Africa will continue to grow unless checked.

Is hybrid technology the answer to Africa's rice woes? Is it worth trying? Although hybrid rice has succeeded in Asia, starting in temperate China and recently in tropical countries, sub-Saharan African (SSA) countries are still skeptical about trying these so-called "smart seeds". Yield data from six Chinese hybrid rice varieties obtained in Guinea last 2003 demonstrated a stunning 9.2 t/ha for the highest yielding hybrid, which is three to six times higher than the yield of local cultivars. However, this first hybrid rice tested in SSA was nonreplicated, with extremely high application of fertilizer (160 kg N, 70 kg P, 70 kg K) and no local variety was included in the trial. Nonetheless, it shows the enormous potential of hybrid rice in Africa.

Using tropical hybrid rice from the International Rice Research Institute (IRRI), the first replicated yield trial was conducted at the Africa Rice Center (WARDA) station, Ibadan, in the 2008 wet season. Twenty-two tropical hybrid rice varieties were tested along with WITA 4—the most popular high-yielding

Glenn Gregorio checks the growth of hybrid rice plants, WARDA, Nigeria.
Photo by O. Adebayo, IITA

Table 1. Hybrid rice yield trial data, IITA, Ibadan, Nigeria, 2008 wet season.

Entry	Yield (t/ha)	% yield advantage over WITA-4	Maturity (d)	Plant height (cm)
IR 83212 H	5.81	27.9	126	118
IR 82391 H	5.43	19.7	123	102
IR 82376 H	5.09	12.3	124	112
IR 83202 H	5.07	11.7	126	120
IR 84711 H	4.93	8.6	124	105
IR 82372 H	4.72	3.8	123	114
IR 81265 H	4.67	2.7	125	103
IR 78386 H	4.54	0.1	123	105
WITA-4 (local check)	4.54	0	127	126

variety in Nigeria—as the local check. Moderate levels of fertilizer (65 kg N, 18 kg P, 18 kg K) and standard cultural crop management practices were applied.

While we were conducting the trial, we received inquiries about the feasibility of growing hybrid rice in Nigeria, Ethiopia, and Guinea. People were excited to learn that tropical hybrids were being tested in the IITA farm. The result of this trial could be the basis for justifying investment in hybrid rice research in SSA.

Some hybrids showed yields that were 0.9–1.3 t/ha higher (20–28%) than the local variety under the same crop management (Table 1). This is good news because to make hybrid rice attractive and acceptable it must have a yield advantage of at least 15–20% over the highest nonhybrid variety. The not-so-good news is that, only two hybrids yielded significantly more than WITA-4. A large number showed poor performance under local conditions since these hybrids were not developed in Africa. The highest yielding hybrid rice yielded less than those tested in Guinea because of the very low fertilizer rate (only 40% of that applied in the Guinea hybrid trial).

This pioneering hybrid rice trial in Nigeria demonstrated that hybrid rice may be the answer to the need to increase rice productivity in SSA. However, creating hybrid rice varieties adapted to local conditions is a challenging process, especially when it comes to hybrid rice seed production. Moreover, the lack of technical skills and infrastructure to carry out hybrid rice seed production programs is a serious constraint.

Hybrid rice development is like a horse race: unless you start, you can never hope to finish the race. Now we have the evidence that hybrid rice technology might work in SSA as it did in Asia. Could this be the vehicle for the Green Revolution in Africa?

WARDA hybrid rice experiments, Nigeria.
Photo by O. Adebayo, IITA

CBSD: Enemy number 1

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A major disease is ravaging cassava production in the lowland areas of Eastern Africa. The culprit is the cassava brown streak disease (CBSD), which has been spreading to higher altitude areas.

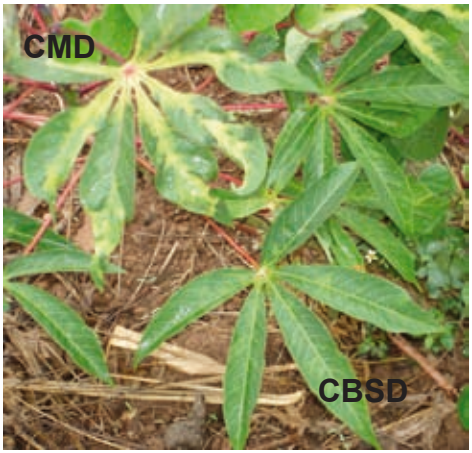
CBSD was first described in detail in Tanzania in the early 20th century and for decades, has been causing severe economic losses in coastal and low altitude Eastern Africa where cassava mosaic disease (CMD) is endemic.

Symptoms

CBSD symptoms are not as distinct in cassava foliage as those of CMD. The main differences are that in CBSD the leaves are not distorted in shape (no epinasty); and the myriad of chlorotic mosaics and blotches in the leaves commonly start as tertiary vein chloroses and are also often very insignificant compared with those from CMD.

CBSD leaf symptoms may be limited to the lower leaves only, depending on the cultivar. If the above-ground symptoms are in the lower leaves only,

Tertiary vein chloroses of CBSD.
Photo by C. Herron, IITA



Two symptoms in the same plant: CMD leaf symptoms are often more obvious and higher in the plant than CBSD leaf symptoms. Photo by C. Herron, IITA

then during plant maturity these often senesce, and thus symptoms can be missed. Other CBSD symptoms in very susceptible cultivars are necrotic longitudinal streaks on the stem (from where the disease derives its name)—which eventually coalesce from the shoot downwards, plant shoot dieback, and petiole necroses.

In the tuberous storage roots of susceptible cultivars, CBSD's dry necrotic lesions may develop from approximately 5 months onwards, depending on the cultivar, with or without any external root symptoms. Root lesions vary widely; in a cross-section of roots these symptoms may range from dry dark brown radially aligned lesions to lesions with a dry white firm interior and a necrotic exterior. The time of first appearance of root symptoms and the tissues in which the symptoms appear are also variable.

Various CBSD root symptoms on cultivars from Tanzania. Photo by C. Herron, IITA

External symptoms in some cultivars appear as radial root constrictions, creating abnormal root shapes. Root symptoms may also be irregularly distributed across fields planted with the same cultivar and also within roots of the same plant. CBSD in the roots can lead to significant reductions in quality and yield, or complete spoilage.

Economic costs

Alleviating the CBSD problem would reduce human burden and toil during the growing season and during processing, and enhance livelihoods.

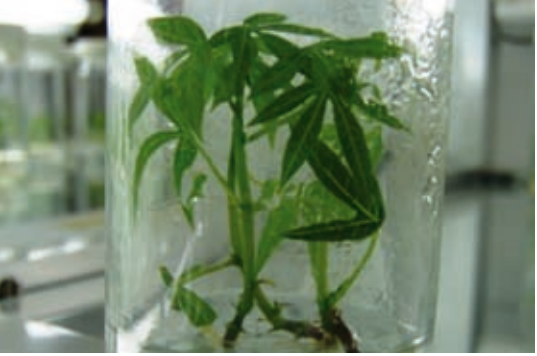
On average, cassava yields are approximately 3.5 t/ha in CBSD-infested fields compared to 10 t/ha in fields without CBSD. The estimated economic loss due to CBSD is about US$130/ha, based on a sample in eastern Tanzania. Extrapolating to the entire cassava-producing regions of the country shows a loss of $45 million/year based on a conservative 14% yield loss from early harvesting to escape heavy CBSD losses. If the farmers' estimated yield loss of 64% at full physiological maturity is taken into consideration, the estimated annual loss for the country is as high as $202 million (Manyong et al. 2008).

Current management strategies

The use of field-resistant cultivars is the recommended disease management strategy. Many field-resistant cultivars should be deployed at any one time in every cassava cultivation area where CBSD is endemic. Susceptible cultivars are not recommended for these areas.

Across the region, many national agricultural research systems (NARS) partners are involved in ongoing cassava breeding programs and multilocational field trials. True seeds from lines of resistant materials identified in CBSD-infested areas (coastal Tanzania) over many growing seasons have been distributed to surrounding countries for incorporation of resistance in farmer-acceptable cultivars. This management strategy has allowed farmers in some of the worst-affected CBSD areas, who had given up growing cassava, to grow and thrive from cassava once more.

Annual monitoring and evaluation of the CBSV presence and CBSV strains over production areas should be combined with this approach. Other management strategies are also being evaluated currently for usefulness, such as use of virus-free propagative materials.



Cassava tissue culture at MARI, Tanzania.
Photo by C. Herron, IITA

Sustaining disease management

Sustaining CBSD management through planting field-resistant cultivars means that scientists cannot afford to be complacent. While these cultivars are proving to be an excellent and the sole current first line of defense against the pathogen, the situation is dynamic, and CBSD damage may be expected over time. This is because genetically diverse pathogens, if allowed to exist widely and in large numbers, can eventually cause damage to earlier tolerant cultivars. Certain pathogen strains or isolates become more "fit" within the population, eventually leading to disease damage or "resistance breakdown".

Ideally, a continuous "pipeline" of cultivars may be needed in every cassava-growing area, together with large-scale detection and monitoring of CBSV strains predominant in the population in any given area on an annual basis. This will provide an early warning detection system for the CBSV strain population shift or resistance breakdown. Long-term management strategies and the development of true resistant cassava cultivars are still desirable.

To date, all new cassava breeding lines tested in Tanzania in field trials have CBSV in many tissues. This indicates that CBSV immunity may not be present within currently used materials. Other attempts must thus be made to provide useful and different mechanisms of resistance to the pathogen. Hybridization between cassava and wild relatives and use of pathogen-mediated resistance could provide various types of resistance to CBSV.

The transformation approach

Constraints with the traditional approach make the use of transformation a viable alternative in incorporating virus resistance to cassava. The Mikocheni Agricultural Research Institute (MARI) under the Ministry of Agriculture, Food Security and Co-operatives, and IITA are undertaking cassava genetic transformation in Tanzania. The project is funded by the Rockefeller Foundation, but will continue to be funded in 2009 by the Bill and Melinda Gates Foundation. Other partners include the Plant Biosafety Office, Tropical Pesticides Research Institute; Agricultural Biosafety Scientific Advisory Committee; National Biosafety Focal Point; and National Biotechnology Advisory Committee.

To date, work on incorporating resistance to the East African cassava mosaic virus and CBSV in cassava cultivars is ongoing. Twenty-six farmer-preferred cultivars from Tanzania that are high yielding but susceptible to CMD and CBSV have been micropropagated. From these cultivars, a total of 1014 plantlets had been produced by end-September 2008. So far, cassava cultivar *Kibandameno* produced the highest number of plantlets (282) followed by *Karatasi* (58), while *Pikipiki* and *Katakya* from the Lake Victoria zone produced the least (4). These tissue culture plantlets will be used for producing embryogenic cultures for transformation.

Reference

Manyong V.M, E.E. Kanju, G. Mkamilo, H. Saleh, and V.J. Rweyendela. 2008. Baseline study on livelihood status and technology adoption levels in Cassava Brown Streak Disease (CBSD)-infected areas of Eastern Tanzania and Zanzibar. Technical Report. IITA-Tanzania. 37 pages.

BEST PRACTICE

Ensuring biosafety

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For thousands of years, developing improved crop varieties has depended on conventional plant breeding methods.

With developments in scientific research and technologies, it is now possible to ”design” crops with improved characteristics within a shorter time and with more precision using biotechnology. Through transformation, genetic information (genes) can be transferred between distantly related species, which would not happen in nature (transgenes). This allows novel and unique characterisrics to be incorporated into crop varieties. Through this technology it is possible to develop new crop varieties (genetically modified organisms, GMOs) with higher yield, adaptation to variable environments, resistance to pests and diseases, enhanced storage time, and improved nutritional values, among others.

Concerns have been expressed about the impact on human and animal health of these transgenes. Concerns also revolve on their possible movement from the bioengineered crop to other cross-compatible crops, and in particular, to wild relatives growing in regions where the crop has its origin or center of diversity, and on the impact of products of transgenes with pesticidal activities on nontarget organisms The concern is that transgenes could confer “fitness” on the crop’s wild relatives, thus making such plants develop into “super weeds,” especially when they become resistant to herbicides. There are also concerns that the protein in the transgenes could be allergenic to people. Outcrossing is a common occurrence between compatible plants and the degree of outcrossing depends on the crop species.

To regulate the release of GMOs to the environment in any country, a set of biosafety regulations are put in place. The International Biosafety Protocol (Cartagena Protocol) places emphasis on the transboundary movements of GMOs and offers a set of guidelines on their safe handling and use. It has been adopted by several countries, including 38 in Africa.

Cowpea plants in the IITA screenhouse. Photo by O. Adebayo, IITA

Biosafety is generally defined as “policies and procedures adopted to ensure the environmentally safe applications of modern biotechnology in medicine, agriculture, and the environment, so as to avoid endangering public health or environmental safety.”

IITA's research on genetic engineering is in consonance with the CGIAR’s guiding principles on the application of modern biotechnology in the improvement of any of its mandate crops. We do not have a separate set of biosafety guidelines. The Institute has worked very closely with agencies of the Federal Government of Nigeria to establish biosafety guidelines for the country. The Federal Ministry of Environment is responsible for regulating the release of bioengineered products, and reports that a Biosafety Bill has been prepared. The document will soon be presented to the National Assembly for deliberation prior to being passed into law. With the existing biosafety guidelines that became operational in 2001, it is possible to carry out research on genetic engineering and test products of the technology under confinement in Nigeria.

Uganda and Tanzania are two countries where IITA is undertaking transformation research. In Uganda, work on transforming banana resistant to banana *Xanthomonas* wilt (R4D Review Edition 1) and nematodes is ongoing (see related story on pages 52–53). In Tanzania, transformation research on incorporating resistance to cassava brown streak disease is being undertaken with partners (see story on pages 16–18). Both Uganda and Tanzania are signatories to the Cartagena Protocol, which requires signatory countries to develop a regulatory framework and the capacity (in terms of people, expertise, and technology) to undertake risk assessments in developing and using GMOs.

The Government of Uganda recognizes biotechnology as a tool that can be used to help stimulate economic development and meet national goals for improving the standard of living for the poor. Biotechnology is specifically included in the Poverty Eradication Action Plan as a component in the Program for the Modernization of Agriculture.

Recently Uganda’s cabinet has approved its first National Biotechnology and Biosafety Policy after 8 years of deliberation. The policy provides objectives and guidelines for promoting and regulating biotechnology use in the country, and contains the guidelines on the legal, institutional, and regulatory framework. The guidelines cover tissue and cell culture, medical diagnostics, industrial microbiology, and biochemical engineering.

For the policy to be implemented, there must be a law. At the moment, a draft bill has been presented to Parliament. The commercialization of GM crops in any country requires this law.

Tanzania released its National Biosafety Framework in 2005. An Institutional Biosafety Committee addresses biosafety activities within any institution conducting genetic modification. The Division of Environment is currently the National Biosafety Focal Point, which is responsible for overseeing the review and approval of applications, and implementation of biosafety issues.

An interim biosafety regulatory process exists for permitting small-scale confined research/field trials of plant and plant products. Applications are reviewed by the Agricultural Biosafety Scientific Advisory Committee and the National Biotechnology Advisory Committee. The Tropical Pesticides Research Institute and the Plant Biosafety Office require risk management measures to ensure that the field trial does not adversely affect the environment or human health.

The first application using the interim measures was for the MARI-IITA project on cassava genetic transformation for virus resistance in Tanzania.

With contributions from Leena Tripathi, IITA–Uganda, and Caroline Herron, IITA–Tanzania.

CGIAR guiding principles

1. In keeping with its mission, IITA will continue to engage in research designed to produce international public goods appropriate for use by resource-poor farmers. In doing so, it will typically use a range of technologies, including in some cases modern biotechnological methods, to produce breeding and planting materials containing traits important to and useful for resource-poor farmers. It follows that IITA believes that genetically modified organisms (GMOs) that contain traits beneficial to small farmers and have been fashioned carefully, with due regard to the range for social, economic, biosafety, and environmental concerns, are a legitimate subject for its research and development.
2. For sound scientific and practical reasons, IITA will continue to work with the gene pools of cultivated species and their wild or weedy relatives as the first and often most effective means of bringing benefits to resource-poor farmers. The formulation of these Guiding Principles is therefore not intended to be, nor should it be interpreted as signaling a shift in emphasis or priorities in IITA research programs: conventional breeding techniques will continue to be used widely in all crop improvement programs. Indeed, they are likely to remain the dominant approach for some time to come.
3. IITA will continue to monitor, research, and assess the possible social and environmental implications of the use of genetically transformed plant varieties in the ecological regions in which they might be used and, especially, in the centers of origin or of diversity of the species that may be genetically transformed. As in other subject areas, these activities will routinely be carried out in cooperation with national agricultural research systems, farmers, and other partners.
4. In all its genetic engineering-related research, IITA will observe the highest scientifically accepted standards of safety in the conduct of laboratory and field experiments.
5. IITA will comply with relevant national or regional biosafety, food, environmental and policy regulations for the deployment of genetically engineered organisms. IITA will not deploy genetically engineered organisms in any country lacking such regulations. In certain circumstances, IITA may voluntarily adhere to higher or more stringent standards than the minimums imposed by national legislation and regulation. IITA will not make GMOs or other such products available in a country without that country's prior informed knowledge, consent, and support.
6. IITA will work with national partners, using the best expertise available, to address potential risks and ensure confidence in the product. If a recipient country lacks the expertise to conduct its own risk assessment, IITA will work with national partners to help develop this capacity, and to develop appropriate strategies and methodologies.
7. Currently, IITA adds a modest number of plant genetic resource accessions each year to those it already conserves under long-term, ex-situ conditions. Under proper management, geneflow between accessions is essentially nonexistent, and thus the presence of GMOs within IITA's collection is not considered to undermine or pose a significant danger to the goal of its long-term conservation of genetic diversity. When circumstances so indicate, however, IITA will screen incoming (and/or already-held) accessions for the presence of promoters or other indications of the presence of GMOs. IITA will make the resulting data available to anyone requesting samples of these accessions, and IITA will take sufficient measures to ensure the appropriate and safe management and use of such materials.

In vitro yam seedlings. Photo by O. Adebayo, IITA

How safe is genetically modified cowpea?

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Genetically modified cowpea resistant to the cowpea pod borer (*Maruca vitrata*) will soon become a reality. But before that happens, IITA is making sure that it addresses some of the potential risks associated with using such genetically modified organisms (GMO). This transgenic cowpea contains the gene from the soil microbe *Bacillus thuringiensis* (*Bt*) that is toxic to the pest.

IITA started preliminary studies to assess concerns, including the development of resistance by the target insect pest to the insecticidal protein expressed in the plant, negative effects of the insecticidal protein on nontarget organisms present in the same agroecosystem, such as natural enemies or pollinators, the accidental introduction of the gene expressing the toxic protein into wild relatives of cowpea (referred to as "gene flow"), and negative effects on human and animal health.

In the meantime, a team of scientists headed by Dr T.J. Higgins of the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia, successfully transformed cowpea with the *Bt* toxin-expressing gene. The transgenic plant has been tested in Puerto Rico but is not yet available for testing in Africa.

IITA started evaluating some of the unintended effects of the purified *Bt*-toxin on nontarget organisms, focusing on natural enemies of the target insect pest, the caterpillar of the pod borer (*M. vitrata*).

Typical damage by pod borer caterpillar boring into a cowpea pod. Photo by M. Tamò, IITA

Parasitic wasp Phanerotoma leucobasis laying its egg into egg of pod borer.
Photo by M. Tamò, IITA

In our first case study, we used a locally available natural enemy, a small parasitic wasp called *Phanerotoma leucobasis*, which develops by destroying caterpillars of the cowpea pod borer. This wasp has a curious biology because it can insert its small egg into the bigger egg of the pod borer, but its immature stages develop inside the caterpillar only when it starts feeding on the cowpea plant. It destroys the pod borer's internal organs from the inside, ultimately killing it.

Following standard protocols in collaboration with Purdue University, USA, we first determined the lethal dosage of the *Bt*-toxin that could kill 50% and 95% of the young caterpillars. Subsequently, we let the wasp parasitize the eggs of the pod borer, and transferred the hatching caterpillars onto an artificial rearing diet contaminated with different doses of the toxin to let them feed on it.

The level of wasp mortality recorded in this experiment favorably compares with results obtained in other studies, and is primarily due to the death of the host caterpillar while feeding on the contaminated diet. Similar experiments are ongoing, using another natural enemy of the pod borer, the exotic parasitic wasp *Apanteles taragamae* introduced into our laboratories from the World Vegetable Center (Asian Vegetable Research and Development Center) in Taiwan.

What would then be the likely impact of *Bt* cowpea on these natural enemies in the field? This question will be definitely answered when real *Bt* cowpea plants will be available for testing in confined field experiments.

For now, we know from previous studies (Romeis et al. 2006) that the negative, unintended effects of *Bt*-transformed crops, such as corn and cotton on natural enemies and biodiversity at large are far less than those caused by repeated applications of synthetic pesticides to control the same pests under conventional crop protection schemes.

For the cowpea pod borer, several alternative host plants exist in the wild where the pest is exposed to the attacks of natural enemies throughout the year, hence providing natural refugia and thus avoiding being negatively impacted by the *Bt*-toxin present in the transformed cowpea.

Exotic parasitic wasp Apanteles taragamae.
Photo by G. Georgen, IITA

Reference

Romeis, J., M. Meissle, and F. Bigler. 2006. Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. Nature Biotechnology. 24:1. p 63-71. January. www.nature.com/naturebiotechnology

Training farmers
using participatory video

Red-podded cocoa, Cameroon. Photo by S. David, IITA

IITA, together with partners, is using an innovative approach to strengthen knowledge of cocoa farmers in Ghana on integrated crop and pest management (ICPM) techniques and practices.

Drama is a popular learning tool in rural Ghana and video viewing booths are found everywhere. Taking a cue from this, IITA's Sustainable Tree Crops Program (STCP) assisted the development of video viewing clubs (VVCs) to train farmers in increasing cocoa production and improving safety and labor efficiency. A VVC consists of 20 to 25 farmers who meet weekly to learn new production practices, using videos, illustrated guidebooks, guided discussions, and field demonstrations.

Using video as a training tool engages the participants, enables new techniques and practices to be shown in a short period, and standardizes the technical information disseminated to farmers.

The VVC is a pilot project assisted by STCP's funding partners including the Chocolate Manufacturers' Association, Nestlé, Sunspire, Mars Incorporated, USAID, and the World Cocoa Foundation.

Project activities are focused on integrated crop and pest management practices in cocoa. Local farmers, cocoa researchers, communication specialists, media specialists, and other partners such as ANS Media, CABI Bioscience, and Stratcomm Africa collaborated in producing the videos. So far, five have been developed, dealing with cultural methods to control black pod disease, chemical control of mirids and black pod disease, pruning, harvesting, pod breaking, fermentation, and drying.

Farmer field school participants. Photos by S. David, IITA

Farmers participated in filming, editing, pretesting, and the final production of the videos, supported by media specialists. Production involved farmers earlier trained in ICPM through STCP-supported farmer field schools (FFS), and specialists. A second group of farmers and communication specialists developed the illustrated guidebooks by identifying technical

Video viewing club in Ghana. Photo by S. David, IITA

messages, depicting these, reviewing drafts, and pretesting the materials.

In recognition of this innovative effort in training farmers, Dr Soniia David, IITA regional participatory extension specialist, and her team at STCP received the 2008 CGIAR Science Awards - Outstanding Communications Category last December.

If farmers adopt the major ICPM practices promoted by VVC, they can increase yield, on average, by 20–40%, and decrease pesticide use by 10–20%. Production training with marketing interventions can also increase household income by 23–55%. STCP expects that at least 60% of VVC-trained farmers will adopt four or more improved practices.

To date, STCP has assisted in training 450 farmers through pilot VVCs in Ghana's central region. It will support partners in training 11,000 farmers in three other West African countries on ICPM over the next five years.

Previously, STCP supported the training of 125,000 farmers in West Africa on ICPM through farmer field schools, a participatory training and research method, to increase production and improve safety and labor efficiency.

Video is becoming increasingly popular as a training tool. The videos developed by STCP can be used for the training of trainers and promoting more effective farmer-to-farmer knowledge and technology diffusion.

STCP will continue to strengthen the capacity of national partners using VVCs and other innovative communication tools to educate farmers.

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TOOL BOX

New genomic tools for improving African crops

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Digital imaging and microscopy: a tool for research and training. Photo by O. Adebayo, IITA

The increase in genomic techniques in the past few decades has thrown the doors of research wide open to agricultural scientists. Conventional breeding has been augmented by various innovative molecular marker-aided techniques. The first wave of molecular marker technology introduced biochemical markers (isozymes and allozymes).

These quickly gave way to the first generation DNA-level markers such as Restriction Fragment Length Polymorphism (RFLP, DNA analysis), Randomly Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), and simple sequence repeat (SSR)—all mouthfuls to the layperson. Those that lend themselves well to automation and multiplexing (use of simultaneous or more than one set of primers in the reaction mix) prevailed because of their cost-effectiveness.

Advances in sequencing technology enhanced the use of DNA sequence-based markers such as SSR and single neuclotide polymorphism (SNP), allowing the development of automated, high throughput (output) genotyping platforms. In a decade, the cost of genotyping has dramatically declined with various techniques developed that allow flexibility under different circumstances. This emphasized the feasibility of molecular breeding.

New tools

Some of the new genetic engineering tools used at IITA include molecular markers for marker-assisted breeding, resistance gene analogs (RGA), Targeting

Induced Local Lesions In Genomes (Tilling), DNA chips, application of DArT markers, and bioinformatics.

In IITA, the development of new genomic tools for molecular breeding and gene discovery is under way for the mandate crops. For instance, new markers have been identified, *in silico* (online), from cassava Expressed Sequence Tags and hundreds of markers validated using a diverse panel of cultivated cassava varieties. After filtering with various criteria, over a hundred new markers were developed, useful for fingerprinting and other molecular genetic applications.

The rapid accumulation of genome sequence data led to the development of an array of functional genomics tools that are being used to understand the complex pathways involved in host plant–pathogen interaction. The RGA technique has applications in cloning, profiling, and host–pathogen interaction.

The RGA technique was used in IITA to assess DNA sequence variation in several elite cassava clones, resulting in several novel sequences, some of which were found to be similar to previously reported RGAs. This information is expected to facilitate the identification of gene-targeted markers for molecular breeding and gene discovery in cassava.

Another new tool is Tilling, a popular technique of reverse genetics for detecting mutations in a target gene, followed by the assignment of phenotypes to the gene sequence. It rapidly gained popularity because it is suitable for automation and for screening thousands of samples. Besides being a non-GMO approach for broadening the genetic base, it provides tools for developing markers for marker-assisted breeding for traits that are cumbersome and expensive to measure.

Tilling work to discover induced and natural mutation in cassava was geared towards specific traits that are intractable (or not easily managed or manipulated) using conventional methods. Adaptation of the technique to other IITA mandate crops such as yam, banana, and cowpea entails the selection of target tissue or organ for mutation, and the selection of similar or different target genes. Crops such as maize and soybean have numerous germplasm resources that can be easily adopted and adapted.

Knowledge of the nucleotide sequences of the target genes is a prerequisite for Tilling. The major IITA mandate crops—cassava, yam, and banana—have very limited genomic resources. To date, nucleotide sequence information for a very few, largely chloroplast, genes could be found in Entrez Gene (http://www.ncbi.nlm.nih.gov/). Investigations in the past decade resulted in the cloning and characterization of expressed cassava genes involved in starch, cyanogen glucosides, and carotenoid biosynthesis. We have used about six full gene sequences (both

A genome-wide 14K DNA chip for cassava (left) and a scan showing 14,000 different genes (right). Photo by IITA.

nuclear and chloroplast genes) and three promoter sequences of cassava for designing primers. However, even in the absence of a nucleotide sequence for the gene of interest, comparative genomics has been successfully used to identify candidate genes. The completion of the genome sequence of poplar and, more recently, of castor bean, is expected to provide useful genetic tools for identifying candidate genes in cassava. Besides, the ongoing cassava genome sequencing (http://www.jgi.doe.gov/sequencing/why/CSP2007/cassava.html) is anticipated to be completed soon, opening a new avenue of research in functional postgenomic studies such as Tilling.

DNA chips have also become popular tools for gene discovery and also for diagnostics. They also provide a reverse genetics tool for identifying gene-targeted markers for molecular breeding. A genome-wide DNA microarray for cassava with ~14,000 probes has been developed at IITA. This is the most comprehensive DNA chip for cassava available to date. This microarray has been used for transcriptome analysis of cassava. Candidate genes that are differentially expressed after virus infection have also been identified.

A cassava DArT chip with 735 polymorphic markers was used to fingerprint a diverse cassava population comprising genotypes from Africa, Latin America, Asia, and breeder lines maintained at IITA. Overall reproducibility of the marker set was very high and average call rate was 97%. DArT markers provide reliable and high throughput molecular information for managing biodiversity in germplasm collections and make rapid genome profiles possible for quantitative trait loci (QTL) mapping.

In vitro plant propagation. Photo by IITA

Bioinformatics

Advances in bioscience technologies such as sequencing, synthesis, imaging, and various other nanoscale assays, have dramatically increased the volume of biological data, which in turn, started the concurrent growth of bioinformatics tools. Bioinformatics is broadly defined as the application of computer technology to the storage, retrieval, and analysis of large amounts of biological information.

The major areas of high-end bioinformatics include the development of databases and algorithms for analyzing and annotating various types of microarray platforms, high-density oligonucleotide chips, variety of mass spectrometry, and diverse platforms of new-generation sequencing data. However, the majority of life science scientists and investigators tend to turn to the Internet to seek end-user web tools and resources (software packages). Countless institutions in the West provide a myriad biological data

resources and services, including expert-curated databases of nucleic acid and protein sequences, data and text mining tools, genome and transcriptome analysis; protein and other macromolecular structure analysis; networks, pathways, and systems biology; evolution and systems biology tools.

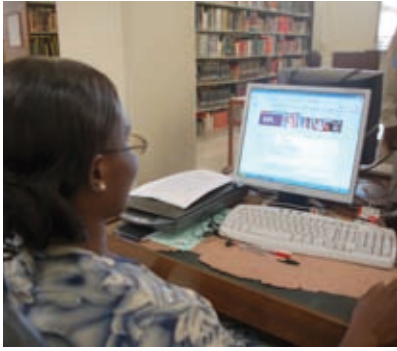
The major tools in the public domain are, however, the development of peer-reviewed, up-to-date, web-accessible databases and web tools (analysis software packages). These resources typically provide an advanced query interface.

The explosive growth of web sites has necessitated that users distinguish between inaccurate personal web sites and reliable resources maintained by a consortium of investigators and/or a legitimate institution. The journal Nucleic Acid Research began to publish annually a collection of molecular biology databases and bioinformatics links directory. The most recent updates of molecular biology databases (http://www.oxfordjournals.org/nar/database/c/) feature over 1000 databases, over 300 of which are on plants, whereas the latest Bioinformatics Links Directory published by the same journal (http://bioinformatics.ca/links_directory/) lists over 1200 links.

Another outstanding issue in the use of online bioinformatics tools is that, as the number of such web resources grows astronomically, even learning how to use the interface is becoming cumbersome, prompting the need for one-stop gateway type of tools for integrated querying (e.g., BioMart, OBRC from the University of Pittsburgh; Bioclipse).

One of the advances in bioinformatics is the availability of programming and scripting languages (Perl, Bioperl, Phyton, and Java) for automating complex but routine steps, such as search, retrieval, and parsing (resolving into and examining component parts) search results. While varieties of commercial integrated analysis packages are available, the cost of initial installation and maintenance becomes prohibitive. Developing our capacity for such routine end-user applications is vital to the support of our molecular biology work.

African researchers working on well-studied crops such as rice, wheat, maize, and soybean will have the best genomic resources at their fingertips, provided that they have Internet connection. To take advantage of publicly accessible web resources,



including the variety of databases, online software, publications, and multimedia learning materials, African scientists and students need institutional support and considerable internal and external funding. As in other fields of science, bioinformatics lags in SSA due, partly, to poor or nonexistent Internet connection. Fast and broad Internet connection is the key to successful online research.

Research in molecular biology is slowly gaining ground in Africa. Any molecular biology research needs to be augmented by a bioinformatics database and online tools.

There is no shortage of available tools for agricultural research or agricultural information and database management. The challenge is in finding the best ones or combinations that suit institutional needs, resources, or preferences. IITA will continue to use suitable and affordable conventional and new genomic tools to undertake research on its mandate crops.

Unraveling cassava problems

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Cassava plays an important role in the food security of many developing nations, especially in Africa, and is also an industrial crop in Latin America and Southeast Asia. Native to South America, cassava was introduced to West Africa from Brazil by Portuguese traders in the 16th century. In the 1700s, cassava was independently introduced into East Africa, also by the Portuguese. Because of its high adaptability to low-fertility soils and its ability to withstand erratic and long periods of drought, cassava is a food security crop and a source of cash for resource-poor farmers. Today, over half of the world's cassava is produced in Africa; Nigeria is the main producer.

As cassava is vegetatively propagated, genetic improvement is arduous because of the high levels of heterozygosity and poor flowering habit in many landraces. Genetic transformation could complement conventional breeding efforts for improving certain traits. Protocols for the genetic modification of model cassava genotypes, such as TMS60444, were first

described in 1996 and have now become routine (Taylor et al. 2004).

Despite this progress, the genotypes that are amenable to genetic modification are not grown by farmers or used as genetic stocks in breeding programs, and this limitd their practical utility and impact at the farm level. Because the current transformation methodologies are highly genotype-dependent and limited to these so-called model genotypes, the genetic transformation of farmer-preferred cassava landraces remains a challenge.

State of cassava genetic transformation

Genetic transformation of cassava was first independently described in 1996 by three research groups. Nowadays, there are at least three modes of cassava genetic transformation, all of which rely on the establishment of somatic embryogenesis.

In one, friable embryogenic callus cultures (FEC) are used to transform and regenerate plantlets. This technology works well with TMS60444 and a few South American varieties, such as MCol 1505. This protocol can yield large numbers of transformants and is possibly the most widely used technology to date. Using this method, several new traits have been introduced into cassava. A major international effort involving research organizations in the USA, Europe, Asia, Latin America, and Africa, aims at enhancing the nutrition of cassava through the genetic modification of TMS60444. Although of African origin, this variety is not used by farmers or breeders as it has unfavorable disease and agronomic characteristics. However, over the past decade, this transformation methodology has provided proof of the concept with genes in TMS60444.

A second procedure relies on transformation of embryogenic cells present

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on immature leaf lobes. Because this technology is faster, it may result in less somaclonal variation than the FEC system since the somatic embryos are not derived from long-term cultures. This methodology also has limitations in terms of farmer-preferred genotypes.

A third methodology uses somatic embryo explants that undergo shoot regeneration (organogenesis). This procedure was first developed by Li et al. (1996) for model cassava genotypes and has been further enhanced by IITA and its collaborator, the University of Copenhagen KU), Denmark, for the African farmer-preferred landraces.

At IITA, somatic embryogenesis has been established for four cultivars: one from West Africa and three from East Africa. These varieties are susceptible to cassava brown streak disease (CBSD) but otherwise have desirable agronomic characteristics. For these four varieties, thousands of somatic embryos (SEs) can be reproducibly generated. Using somatic embryos as source explants, transgenic *Tokunbo*, a landrace cultivated in several countries in West Africa, has been produced using a beta-glucuronidase (GUS) gene construct.

Enabling technologies

In addition to genetic transformation protocols, a set of other technologies is required for biotechnology-mediated cassava improvement. Since many traits target specific tissues, such as roots, a wider range of promoters or other regulatory elements needs to be investigated and tested in cassava to achieve optimal and stable transgene expression in these tissues or in the whole plant. The aspect of stable gene expression, i.e., the absence of gene silencing, is especially relevant for a vegetatively propagated crop, such as cassava. The international effort to sequence the cassava genome as well as initiatives of several labs, including IITA, to develop functional genomics tools will greatly expand our tool set for biotechnology-mediated cassava improvement, whether through transgenesis or marker-assisted breeding approaches.

Transgenic cassava Tokunbo in the greenhouse. Photo by IITA

Many biotechnologies are protected by intellectual property rights and thus are not always freely accessible, especially to research institutions in the developing world. In this context, a new plasmid was developed at IITA for *Agrobacterium*-mediated genetic transformation of cassava and other dicotyledonous plants. This plasmid contains a Cassava Vein Mosaic Virus promoter cassette and was tested in tobacco and cassava using a beta-glucuronidase reporter gene. High, constitutive expression was obtained in leaf, stem, and root tissues of both crops, thus showing that the new promoter cassette was fully functional.

A derivative of this plasmid was made by IITA's partner, the

The blue color shows expression of a reporter gene in leaf, root, and stem of the cultivar Tokunbo, a transgenic African cassava. Photo by IITA

German Resource Centre for Biological Materials (DSMZ) to manipulate gene expression via RNA interference. RNA interference is widely used in animal and plant cells to down-regulate gene expression. In cassava, this approach is currently being used to engineer virus-resistant plants and plants that produce reduced levels of cyanide.

Beyond genetic transformation

One of the highlights at the International Congress of Genetics, held in Berlin in 2008, was a report on the use of artificial mini-chromosomes in maize. The methodology makes use of artificial mini-chromosomes that can replicate and are stably transmitted over several generations, in a way similar to natural chromosomes. These mini-chromosomes contain DNA sequences found in centromeres, the chromosomal regions needed for inheritance. Rather than inserting new genes randomly

into a plant's natural chromosomes, as happens in genetic modification, these mini-chromosomes remain separate. One of the advantages of this technique is that it allows multiple genes to be arranged in a defined sequence with their own regulatory sequences, resulting in more consistent gene expression. So far, this technique has been used only in maize but it can potentially be applied to other crops as well, including cassava.

Outlook

Although methods for cassava genetic transformation were developed over a decade ago, genetic transformation of farmer-preferred (African) cultivars has only recently become a research priority. IITA and its partner KU in Denmark have recently produced a first transgenic cassava variety called Tokunbo which is cultivated in Nigeria and some other West African countries.

As a complementary approach to cultivar-specific transformation, the movement of transgenes through conventional breeding should also be considered and, ideally, also artificial mini-chromosomes, the new gene-transfer technology established in maize.

As new and cost-effective DNA sequencing methodologies are becoming mainstream, the amount of genomics and genetic information for cassava will vastly increase in the near future and greatly expand our tool set for biotechnology-mediated improvement. From a technical standpoint, it is realistic to expect that new varieties, in part produced using biotechnology tools, will become available in the near future.

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Christian A. Fatokun:
the guardian of biosafety

Dr Christian Fatokun is a plant breeder/molecular geneticist in charge of biosafety at IITA. He attended the University of Ibadan where he obtained BSc and PhD degrees in agriculture and plant breeding, respectively. He was a lecturer in plant breeding and genetics at his alma mater before joining IITA as a scientist in September 1993. He was also a postdoctoral research fellow in IITA's farming systems program between 1976 and 1977. According to him, his active working career will end at IITA when he retires this year.

Tell R4D Review about your work.

I work on cowpea improvement. In Nigeria, we eat a lot of cowpea, a cheap source of protein. Before joining IITA I had worked on exploring the genetic potentials of wild cowpea relatives and on the genomics of cowpea. I continued research in both areas when I joined IITA. Now my research is on improving cowpea for enhanced levels of drought tolerance. Compared with many other crops, cowpea is drought tolerant. Our efforts are geared towards increasing the level of tolerance in existing improved and farmers' varieties. The new varieties would be better adapted to drought stressed environments.

What are some of the highlights of IITA's work on cowpea?

IITA has a global mandate to study cowpea. It has conducted research, including agronomy, pathology (bacteriology, virology, nematology, Striga and Alectra biology), entomology, physiology, microbiology, socioeconomics, technology transfer, seed storage of germplasm lines, breeding and genetics, and attempts to genetically modify the crop.

Making wide crosses, studying the genome structure, identification of markers with effects on traits of importance, and evaluating germplasm lines for their drought tolerance are all aimed at genetic improvement of cowpea.

We are now developing varieties with enhanced drought tolerance, resistance to *Striga* and *Maruca vitrata*, and possessing consumer-preferred traits, such as higher nutritional levels and easier processing. The *Bacillus thuringiensis* gene which was recently introduced, through bioengineering, into cowpea in Australia can be transferred, using conventional breeding techniques, to lines that are popular with farmers and consumers if the gene is found to be effective in controlling *Maruca*. I foresee increased research on the contribution of cowpea consumption to human health.

What impact does IITA's work on cowpea research have on producers and consumers in Africa?

The average cowpea grain yield for Africa up to the 1990s was around 300 kg/ha. According to FAO, average cowpea grain yield for West Africa in 2006 increased from 240 kg/ha to 470 kg/ha. For Nigeria, the world's number one producer and consumer, grain yield increased from about 470 kg/ha to 670 kg/ha. IITA research on cowpea contributed to these increases.

There are now new varieties that mature within 60 days from planting. These varieties suffer less damage from drought and pests than traditional varieties that remain much longer in the field before flowering and setting pods. Some improved breeding lines with resistance to field pests such as aphids and flower thrips and the parasitic weeds, *Striga gesnerioides* and *Alectra vogelii* are also available. Farmers who adopt these new improved varieties apply insecticides less often. These have reduced production cost, less damage to the environment, and provide less exposure to chemicals. Improved production practices have also increased yield. Improved dual-purpose varieties are particularly beneficial to farmers in the dry savanna regions because the haulms are a very good source of quality fodder for livestock.

What is your work philosophy?

It is to do my very best and leave the rest for others along the chain of workers. The primary school that I attended has as its motto: "what is worth doing is worth doing well". We were made to repeat this statement regularly.

In addition, I am convinced that the most benefit can be derived from one's income only when one always has put in one's best efforts. Essentially, the nature of the harvest accruing to an individual cannot be different from what has been sown.

How did you get involved in biosafety work?

I participated in the preparation of the Nigeria Biosafety Guidelines that IITA initiated in 1993 and published in 1994. Later, I was invited by the Federal Ministry of Environment to participate in preparing the National Biosafety Framework.

IITA has a biosafety containment facility built next to the biotechnology laboratory. It is at a biosafety level (BL2-P standard), which is appropriate for growing bioengineered crops. We also have a confined field trial site on campus at Ibadan that has not been used since it was constructed. IITA is therefore committed to ensuring compliance with international standards in biosafety.

Fatokun shows off cowpea plants in the screenhouse. Photo by O. Adebayo, IITA

What are some of the biggest constraints to the adoption of biotechnological tools or products in Africa?

The use of these relatively new tools in research in Africa is in its infancy. There is great promise in the new technology. However, the capacity to carry out research using these new tools is very limited in most African countries. The critical mass of scientists in the field is nonexistent in several African countries. In addition, the needed infrastructure is also lacking. A few countries, such as South Africa and Egypt, have been exemplary in developing scientific capacity and providing resources to support research in this field. Their

farmers, both commercial and small-scale, have seen some of the benefits that the technology has to offer. Burkina Faso has recently joined these countries to commercialize a genetically modified crop. Kenya has likewise given approval for the growing of a bioengineered crop in the country.

Biosafety regulatory regimes would be needed for the technology to prosper in Africa.

What could be done to take advantage of opportunities that current agricultural technologies provide and harness them for the development of African agriculture or the improvement of food security in SSA?

Governments in Africa need to show more commitment to agriculture by addressing the problems that limit productivity. For example, crop production is mostly rainfed and this exposes crops to terminal drought, especially in the savanna region where there is large potential farmland. There are very limited irrigation facilities where year-round farming could be practiced.

Inadequate fertilizer supply to farmers is a major problem. Many of the newly developed crop varieties are responsive to good soil fertility. They cannot attain their optimum yield where soil fertility is low. There should be commitment on the part of governments to ensure availability of this important input. The private sector should be encouraged to get more involved with production, importation, and distribution of agricultural inputs.

In a nutshell, expansion of irrigation facilities, better supply of fertilizers and other needed inputs, such as improved seeds, etc., and the construction of motorable roads to ensure easy transportation of farm produce would, among others, enable farmers to take advantage of available technologies. If these and other identified problems faced by farmers could be addressed, agricultural productivity would be increased and food security ensured.

When farmers come across good technologies, they embrace them.

Any lessons or insights that you want to share with colleagues?

We should see ourselves as members of a community of scientists working to solve problems of food security and poverty in Africa. There is a great need for harmoniously working together since we are all working toward the same objectives of increased food production, ensuring food security, and regeneration of income for our clientele. The Yoruba people in Southwestern Nigeria have a saying. When translated, it means that if hunger is eliminated from among the problems associated with poverty, then the burden of poverty is remarkably reduced. As IITA staff, we should always remember that our little individual contributions add up and could make a difference to many people.

Cowpea plants. Photo by IITA

Maria Ayodele:
Invest in people

Dr Maria Ayodele is from Cameroon. She set up and has been in charge of IITA's Germplasm Health Unit (GHU) since 1998. IITA recognizes that germplasm health is a very important concern, and is proactive about ensuring the production of good quality and healthy plants by guarding against the introduction of exotic seed-borne pests, and preventing their spread to collaborating countries and partners. The GHU has thus adopted strict phytosanitary measures and has facilitated the movement of thousands of items of germplasm materials for its mandate crops every year.

Dr Ayodele obtained her first degree in the Netherlands on tropical agriculture, MSc in plant bacteriology from the University of Aberdeen, Scotland, and PhD in plant pathology, University of Ibadan, Nigeria. She also has a Diploma in Bible Studies and a Certificate in Discipleship, and is an Assistant Pastor of the Redeemed Christian Church of God. Dr Ayodele is a mother of six and several other children in the Lord; she specializes in mothering and welfarism.

Please tell R4D Review about yourself.

I am a plant pathologist by training, specializing in bacteriology and mycology (fungi), but also in seed pathology, phytosanitary regulations, and capacity building. I take care of plant health testing and diagnostics, and liaise with breeders and other scientists for test results, and with national partners for phytosanitary requirements and plant quarantine.

Please describe your work. What is your main research interest?

As a research support scientist, I help IITA in testing seeds for import or for sending to partners by making sure that they are disease-free to prevent the spread of exotic pests and diseases. I do plant health testing and grow seeds or other plant parts, such as leaves, stems, or tubers, in a containment facility; I inspect the plants in the field or in the genebank; take care of the bacteriology and mycology screening; send the materials to the Virology Unit for viral testing; compile all the test results and send them to the scientists; and make sure that all the proper documentation in terms of phytosanitary permits or requirements are provided for each crop and for each cooperating country.

I liaise with the plant protection and quarantine service organizations of partner countries where IITA sends or imports seeds or other plant materials for use in research. With FAO, I provide technical backstopping in plant health and phytosanitary regulation, and also capacity building for SSA partner countries, including Nigeria, Bénin, Burkina Faso, Gabon, Gambia, Guinea Bissau, Mali, Togo, Cote'Ivoire, Niger, and Senegal. For example, since some partners do not have the capacity for plant health diagnosis, IITA works with the Plant Quarantine Service (PQS) or the national plant protection organizations in doing the testing, with the PQS doing their own inspection. Otherwise, I travel to where the test plants are and inspect them.

Last year, I was part of a team that conducted training on pest risk analysis and the safe movement of germplasm for partners in the national programs of Tanzania, Zanzibar, Uganda, Kenya, and Zambia, Burundi, Malawi, and DR Congo.

I also do some research, specifically in the areas of classification and characterization of anthracnose for yam, morphological characterization of gray leaf spot in maize,

and the establishment of pest-free areas for multiplying germplasm materials.

What are some of the highlights of your career at IITA?

I have enjoyed the capacity building part—coordinating training and sharing my knowledge with partner-participants and investing in people. I maintain links with participants who start as students and later become colleagues, and keep communication lines open. They can always come back to me with questions. My relationship with them is based on honesty and mutual respect. This approach has helped such that I have never had any difficulty when asking for plant quarantine documentation.

What is your work philosophy?

I believe that people should be happy with what they are doing, or not do it at all. I like my job, I like what I am doing, and I am happy transferring knowledge and building the capacity of partners in plant health testing and diagnostics.

It is important to me that the type of job that I do motivates me. That way, I get complete satisfaction. I want to see that the client is happy, so it is important to work together with clients—work with people, work with results; in short, be self-motivated. Getting good results makes people happy, and I make sure that I deliver on those results.

I am most happy when I am in the field—I see the challenges there—and they make me think and look for solutions to problems; for example, why are this year's plants different from those of last year? Was it the climate?

What lessons or insights do you want to share with colleagues?

I work a lot with partners in the national programs and this is very challenging because of differences in capacities. When I work with my "students", I usually break down information and bring it to their level. This means simplifying language to make science, even common concepts, understandable. I provide hands-on exercises so participants are exposed to the practical side; for example, I bring them to the field to do actual disease diagnosis.

I am now working on a practical manual on field diagnosis for each IITA crop. This is intended for students of agriculture, universities and colleges, extension workers, farmers, and partners. Our scientists should be encouraged to produce simple monographs on their research breakthroughs, documents that are easily affordable and accessible to our clients. At the moment, our scientists write mainly for academic journals. Of course, we know that many of our clients have no capacity to pick up information in those journals.

When I approach work, I do not look only at the problems. Yes, I find out what the weaknesses are, but I focus on the strengths and think of solutions. I use this approach for everything. Not everything can be bad. Negativity is a wrong thing in life, so it is best to find the positive aspects in people or situations. Once you get a working system, look at what needs to be changed. Oh, and do not criticize—be constructive.

Lastly, we should also be resourceful and show our initiative at work.

You would be retiring from IITA soon. What would you want colleagues (or partners) to remember you by?

I want colleagues or partners to remember me as a good teacher and effective communicator—a colleague who is results-oriented, or who works until she gets results. But you should be asking my colleagues about this, not me!

What do you wish for Africa?

My wish is for Africa to have the phytosanitary structures in place where feasible, to prevent the introduction of exotic pests and diseases that are dangerous to African crops, and to assist and sustain agricultural development for food security and the prevention of genetic erosion. We can sustain agriculture in Africa if we protect it by preventing the introduction of pests accompanying plant imports—unintentionally introduced—and avoiding the spread and establishment on alternativee hosts.

Would you like to share some personal details about yourself?

Although I am an extrovert, I am a very private person. So, take what you see, and whatever you don't see, don't bother to look for it.

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Steffen Abele:
Juggling science and research management

Steffen Abele is a Director of Research whose job is to facilitate the scientists' research in terms of managing regional activities, and in IITA's programs, such as in Banana and Plantain systems, Opportunities and Threats, and Agriculture and Health. He is a socioeconomist based in IITA-Tanzania, where the East and Southern African hub is being established. This regional hub will provide administrative, communication, information technology, and more importantly, research support and more efficient delivery of services to the critical mass of IITA scientists based in the region.

How has it been like working for an international organization like IITA that is focused on finding solutions to development problems in Africa?

This is one of the best jobs a scientist can think of: conducting interesting research and at the same time finding solutions to help people in Africa overcome hunger and poverty. And all of that in the most interesting place in the world: Africa.

As an economist, how do you measure the impact of IITA's R4D on, for example, banana and plantain?

We are only at the beginning of measuring the impact of research, ours and our partners. Banana and plantain are some of the most important food and cash crops in Africa, and our studies already show that the impact we are having from our research brings hundreds of millions of dollars annually into peoples' hands, in particular in East and Central Africa.

As head of the Banana and Plantain Systems Program, what are your plans in the medium term? In the long term?

We—I am not at all working as a lonely "head of program", but in a very dynamic and enthusiastic team—are trying to raise the profile of banana and plantain and their potential for food and cash. A good step forward was the Banana Conference in Mombasa in 2008. I hope that next year, we will look at an even better program, that is consolidated in the most important places in Africa for banana and plantain, with efficient and innovative research being conducted.

Where do you see banana and plantain improvement heading?

In the coming years, we will spend a lot of effort identifying and managing present and emerging diseases. This will be done through conventional breeding and genetic modification of germplasm, but also through new disease management practices, and work on healthy planting material, for example, through micro- and macropropagation, also through planting material sanitation. Beyond that, we are also learning a lot in the field of agronomics at the moment, and we hope to have a lot of good recommendations for crop management soon, which will raise yields further.

How could banana and plantain be commercialized in Africa? What are some of the problems in commercializing the crop in SSA?

Banana and plantain are already an important cash crop in Africa. Yet farmers suffer from problems that affect these crops more than any other crop. The crop is perishable and therefore difficult to transport and market. African banana are not competitive in the global market. We will tackle these problems through a multitude of measures: optimizing crop management according to market needs, providing market information, and producing clean planting material—which is a market in itself.

How would you encourage colleagues at work?

Keep going, even if it's tough! You will succeed!

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Woman selling yam in Bodija market. Photo by O. Adebayo, IITA

Jacob Hodeba Mignouna:
leading the way in science

Jacob Mignouna is a molecular biologist/biotechnologist with an MSc degree in chemical engineering and a PhD in molecular biology and genetics, both from the Catholic University of Louvain, Belgium.

He joined IITA in 1992, as a research scientist-biotechnologist. He led the Biotechnology Unit and developed and implemented a research program on the use of molecular genetic tools to improve food crops and efficiently manage crop genetic resources.

He was a distinguished Frosty Hill Research fellow and had worked as visiting scientist at the Institute for Genomic Diversity, Cornell University, Ithaca, New York; Research Associate Professor of Biotechnology and co-Director of USAID's Farmer-to-Farmer program in East Africa at Virginia State University, Petersburg, Virginia; and Biosafety Consultant for the USAID Program for Biosafety Systems (PBS), International Food Policy Research Institute (IFPRI), Washington D.C., USA.

As Technical Operations Manager at African Agriculture Technology Foundation (AATF), he identifies opportunities for agricultural technology interventions, assesses the feasibility and probability of success of project concepts, identifies sources of appropriate technologies, negotiates their access and deployment, and provides overall leadership in the implementation of AATF's project portfolio.

Please describe AATF's work and your work.
AATF is a not-for-profit organization that facilitates and promotes public-private partnerships for the access and delivery of appropriate proprietary agricultural technologies for use by resource-poor smallholder farmers in sub-Saharan Africa (SSA).

The Foundation is a one-stop-shop that provides expertise and know-how that facilitates the identification, access, development, delivery, and use of proprietary agricultural technologies.

AATF works toward food security and poverty reduction in SSA, and its structure and operations draw upon the best practices and resources of both the public and private sectors.

It also contributes to capacity building in Africa by engaging African institutions to work in partnership with others.

AATF strives to achieve sustainable impact at the farm level through innovative partnerships that bring together players all along the food value chain—from smallholder farmers to national agricultural systems, regional and international research organizations, and technology developers.

Currently, AATF is working on biotechnology projects focusing on maize, cowpea, banana, rice, and sorghum—all important crops in Africa. We are also looking at ways to address aflatoxin contamination in peanuts and cereals and processing of cassava.

How did the AATF and IITA partnership come about?
In 2004, IITA approached AATF seeking to access candidate genes conferring resistance against banana bacterial wilt (BXW). IITA had already established contact with Academia Sinica, Taiwan, which held patents to the technology and wanted AATF to negotiate for a license to the ferrodoxin-like protein

(*pflp*) and hypersensitive response assisting protein (*hrap*) genes from the institute.

In August 2005, IITA, Uganda's National Agricultural Research Organisation (NARO), and AATF convened a two-day consultative meeting at which stakeholders, including other national research institutes from the Great Lakes region, including IRAZ and other NARS in the region, drafted a project concept note on developing banana bacterial wilt-resistant germplasm.

Soon after, AATF approached Academia Sinica, Taiwan, to license the *pflp* and *hrap* genes to it on humanitarian basis.

The initiative has since grown into a full-fledged project designed to enable smallholder farmers in Africa have access to disease-resistant high-yielding banana developed from East African highland varieties.

The project has two components. One focuses on developing transgenic varieties using the acquired technology and the other on improving the capacity of institutions in the region to produce high-quality disease-free planting materials using tissue culture technique.

AATF coordinates the project, including providing support in management of intellectual property rights and regulatory issues, while IITA leads the research, working with Academia Sinica and various institutions, including NARO-Uganda and IRAZ (the national research institution of Burundi), and public and private tissue culture laboratories in Kenya, Tanzania, Uganda, Burundi, Rwanda, and DR Congo.

Through the collaborative research, five banana cultivars—*Kayinja*, *Nakitembe*, *Mpologoma*, *Sukali Ndizi*, *and Nakinyika*—have been transformed using an *Agrobacterium*-mediated system. Several transgenic lines have been produced and tested in vitro by artificial inoculation with the pure *Xanthomonas campestris* pv. *musacearum* (Xcm) bacterial culture. Some of the promising lines showed no bacterial wilt symptoms. These plants were further analyzed and confirmed to have the transgene *pflp* integrated into the banana genome.

With progress on banana transformation well under way, AATF will soon commission a biosafety study. The findings will inform stakeholders as they develop a roadmap for the various processes required for regulatory approvals as the project progresses through the product development pipeline.

Please share your insights on collaboration and partnership.
First, collaboration works well if there is a clearly articulated and shared need for joint effort.

Secondly, such partnerships work best if roles and responsibilities are well defined. Work in the banana project is governed by a Memorandum of Association that recognizes the capacities of the partner organizations and facilitates each to contribute optimally to the project.

Also important is the need to bring on board potential partners early enough so that they can provide their input into the project design right from the concept stage. In this project, and generally in all AATF initiatives, we have found comprehensive consultations with a wide range of stakeholders, especially at the formative stage to be a critical success factor.

Third is information flow. Building a communication strategy into the project design ensures that the information needs of partners and external stakeholders are adequately met.

Preparing cassava leaves for silage.
Photo by S. Kolijn, IITA

Capacity building is core to all AATF partnerships because of the key role it plays in moving the technologies through the entire food value chain, including scaling up of technologies. In this project, the hub of banana transformation work is at Kawanda, where IITA researchers are working with scientists from national research systems and jointly carrying out the transformation work. This kind of collaboration ensures that staff of national agricultural research institutes in the target countries provide continuity of work in their home country.

Another important aspect of partnership is focus on the smallholder farmer. We have found that having a shared commitment to improve the livelihoods of resource-poor farmers—a clear statement about the ultimate focus of our work—enhances stakeholders' commitment to project activities.

Then, of course there is the need to have clear negotiated ways to deal with conflict, ensure accountability, and other governance issues.

How did AATF handle the licensing agreement for using the genes for developing resistance to Xanthomonas wilt in bananas?

AATF typically follows a strategy in which it takes the role of the principal and "responsible party" in facilitating public-private partnerships. AATF has entered into licensing agreements to access and hold proprietary technologies and to ensure freedom to operate (FTO) for all the components of the technologies. The Foundation then sublicenses partner institutions to carry out research and adapt technologies for regulatory compliance, and to produce and distribute the technologies. After signing the relevant agreements allowing use of the technology, AATF and partners are guided by a business plan that spells out the roles of each partner and how the technology will be used.

As the principal party, AATF monitors compliance with the requirements of sublicenses to minimize the risk of technology failure, and facilitates the work of appropriate partner institutions to ensure that links in the value chain are connected and result in technology products that reach smallholder farmers.

How would this research impact on banana producers and consumers in Africa?

Millions of people across the East African highlands depend on banana for their livelihoods, directly for food and smallholder producers for the market or as traders and other players in the crop's value chain. Since banana *Xanthomonas* wilt broke out in the region, it has caused losses estimated at over US$500 million in Uganda, eastern DR Congo, Rwanda, Kenya, and Tanzania.

Scientists interviewing cassava farmers. Photo by IITA

In parts of Uganda, where the crop is a staple, some families reported that their banana production had decreased by up to 80%.

Given the severity of losses caused by BXW and the fact that the effectiveness of existing remedies is limited, development of disease-resistant varieties will have a huge impact on livelihoods. The benefits can be multiplied many times over by making available clean planting materials to enable farmers to rapidly expand their production.

Increased production will lead to higher incomes for families from sale of the crop, including to the vastly untapped European and American markets, now dominated by South American countries, which account for 60% of the global banana trade.

How do you see the use of molecular genetic tools or biotechnology research advancing in Africa? What are some of the biggest constraints to adoption of biotechnological tools or products?
I believe that properly applied agricultural biotechnology holds the key to food security in Africa. Molecular genetics tools should be used not only to improve crops but also to create a better understanding of the abundant diversity of African genetic resources for food, feed, medicine, etc.

The biggest constraints to adoption of biotech tools include limited resources—both infrastructural and in terms of trained scientists and other personnel.

Some African countries also lack a regulatory environment conducive to biotech research and development. Although there have been positive changes over the past couple of years, a lot more needs to be done in these areas, including developing regulations to operationize biosafety laws.

What could be done to take advantage of opportunities that current agricultural technologies provide and harness them for the development of African agriculture or the improvement of food security in SSA?
There are various ways but a key one is by building partnerships, such as those AATF facilitates, that can help access needed technologies, move them from product development and into the hands of farmers. This means different organizations working together to identify and resolve farmer constraints through pooling of available resources where necessary.

We also need to rapidly enhance our capacity to use biotech research. African governments and institutions need to come together and harness their various strengths to develop biotech infrastructure on the continent.

This means training more high-level scientists, equipping laboratories that can serve as centers of excellence and strengthening collaboration among African institutions and between them and research centers and universities abroad.

Lack of awareness about biotech is a major challenge. There is a need for well-designed communication campaigns not only to increase awareness and knowledge of biotechnology, but to increase public acceptance and use of technologies.

You used to head the Biotech Unit at IITA. Please tell R4D Review about your experiences in using biotechnology tools then.
The focus of the Biotechnology Unit, which comprised seven scientists and 45 support staff, was to use the tools that were then available for improving IITA mandate crops. Our work was mainly in two areas. One was developing genetic markers for the characterization of genetic resources, molecular breeding for pests and disease characterization, and exchange of germplasm. The other area was genetic engineering, where we applied tools to address intractable pests and diseases, such as insects that affect cowpea, viral and fungal diseases affecting plantain and banana and cassava mosaic disease. We also addressed diseases and pests in yam, another important food.

What are your aspirations for Africa?
My vision is to see Africa embrace all available tools, including biotechnology and develop the capacity to use them to produce enough food and improve the livelihoods of communities across Africa.

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Farmers transporting cowpea harvest. Photo by S. Muranaka, IITA

Designer (cowpea) plants

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Plants can be designed to order. Science has long found a way to combine good and useful characteristics in a plant by studying the genes for such traits, and putting them together in a process called “genetic engineering.”

Cowpea is grown mainly for its protein-rich grains and quality fodder for livestock. At present, biological control and conventional breeding methods are proving inadequate in developing cowpea varieties resistant to destructive pests, such as the legume pod borer *Maruca vitrata*.

M. vitrata is the most widespread cowpea pest. The adult moth lays eggs on the plant. The larvae that emerge from the eggs damage plants in the field, particularly during the reproductive stage, through feeding on young succulent shoots, flowers, pods, and seeds. This pest can cause significant grain yield reduction, between 20% and 80% if not controlled with insecticides.

Farmers usually spray insecticides to protect the cowpea crop from *Maruca* and other pests. Purchasing chemicals, however, adds to the production cost, thus reducing the farmers' profit. Also, farmers are not well equipped to protect themselves when using such toxic chemicals. In some farming communities, adulterated chemicals that do not control the pests are sold to farmers. The development of cowpea varieties with resistance to *Maruca* and other insect pests would benefit the most resource-poor African farmers who grow the crop.

Cowpea is grown extensively in the savanna region of sub-Saharan Africa (SSA). At least one major insect pest attacks cowpea at every stage in the life cycle, including seeds in storage. These pests are significantly responsible for the low grain yield in farmers’ fields. Through conventional breeding, some varieties have been developed that show resistance to some of the pests, such as aphids and flower thrips, and low levels of resistance to the storage weevil. However, not much progress has been made in host plant resistance, especially *M. vitrata*.

Efforts continue to identify parasites and predators that could be used as biocontrol agents. When deployed, such agents would greatly reduce the population of the *Maruca* larvae in the field, giving the cowpea plant some respite for the production of flowers and pods containing whole and well-formed seeds.

Using conventional breeding, several hundreds of accessions of cultivated cowpea and its wild relatives have also been screened for resistance to this pest. Accessions belonging to *Vigna vexillata* were found to be resistant to *M. vitrata*. These accessions were found to be closest to cowpea in a phylogenetic study of diversity in the *Vigna* species. The study was based on data obtained after DNA genotyping. Efforts were made to cross cowpea with *V. vexillata* but without success.

This strong cross-incompatibility makes gene exchange between the two species impossible. This is where biotechnology comes to the rescue. Two major steps are needed to develop genetically modified cowpea with resistance to *M. vitrata*. First is developing a transformation system and the second

Pod borer caterpillar boring into a cowpea pod. Photo by S. Muranaka, IITA

is identifying the transgene that would be effective against the pest when introduced into cowpea. Since *Maruca* is a Lepidopteran, some of the genes from *Bacillus thuringiensis* (*Bt*) should be effective against the insect's larvae. IITA screened several *Bt* protoxins on *Maruca* by incorporating different concentrations in the diet fed to the larvae. The protoxin of *Bt* gene "Cry1ab" was found to be most effective even at very low concentrations in the artificial diet. This *Bt* gene (Cry1ab) was therefore selected as the candidate gene for designing *Maruca*-resistant cowpea.

Scientists at the Commonwealth Scientific and Industrial Research Organization (CSIRO) in Australia developed the transformation system using an IITA-developed breeding line "IT86D-1010" derived from a cross between "TVx4659-03E" and "IT82E-60". CSIRO scientists have now obtained cowpea lines containing the *Bt* gene. Monsanto donated the *Bt* gene used to transform cowpea by licensing it to the African Agricultural Technology Foundation (AATF) for use in Africa. The Rockefeller Foundation and USAID funded this cowpea transformation project.

The *Bt* cowpea had been tested in the CSIRO laboratory in Australia and found to be effective against the larvae of another Lepidoptera, *Helicoverpa armigera*. The *Bt* gene in cowpea is expected to be effective against *M. vitrata*, the cowpea pest, but needs to be tested in an environment where *Maruca* thrives. Apart from Burkina Faso, none of the African countries where cowpea is an important crop has a biosafety law in place. A few lines of the *Bt* cowpea were, therefore, taken to Puerto Rico for field testing. The field trial was carried out in late 2008. If the *Bt* gene in the cowpea lines is found to be effective against *Maruca*, the next step would be to transfer the *Bt* gene into popularly grown cowpea varieties selected from interested countries. The line presently containing the *Bt* gene is not high yielding, and farmers are not likely to accept it readily.

Under the international biosafety protocol (Cartagena protocol on biosafety) it is necessary to carry out risk assessment on the *Bt* cowpea before it is introduced to another country. The data obtained from risk assessment form part of the dossier that accompanies applications requesting for importation to any country. Risk assessment would entail studies on gene flow, the effect of the transgene on nontarget organisms, food safety, and resistance management strategies (see related story on pages 22–23).

A meeting of experts in these various fields is planned in March 2009 at the Donald Danforth Plant Science Center, St. Louis, Missouri, USA. The experts would design studies to address the different questions that may arise from biosafety regulators in the countries where the *Bt* cowpea is meant to be grown. Many of the proposed studies are necessary, because cowpea is an indigenous food crop in SSA where cross-compatible wild relatives are found growing in agroecologies similar to farmers' fields. Biosafety reviews in the African countries would, therefore, be rigorous.

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 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.

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