Endophyte-enhanced banana tissue culture: technology transfer through public-private partnerships in Kenya and Uganda

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Abstract

The lack of clean planting material is a major constraint for banana production in East and Central Africa. When establishing new fields, tissue culture plantlets will reduce damage by banana pests and diseases. Pest infestation or reinfestation, however, remains a vital concern. Fungal endophytes, when inoculated into banana tissue culture plants, extend the benefits of clean planting material. Endophyte-enhanced tissue culture technology is being developed at the International Institute of Tropical Agriculture (IITA). Tissue culture production facilities in Uganda are in their infancy, while in Kenya the situation is more developed. Public-private partnerships between IITA and Agro-Genetic Technologies Ltd (Uganda), and Jomo Kenyatta University of Agriculture and Technology (Kenya) have recently enabled IITA's project to make great progress towards bridging upstream research and downstream technology transfer. Additionally, unexpected synergisms have emerged through mutual exchange of information and experience. Based on IITA's highly positive experience, such public-private partnerships should be introduced as early as possible in the developmental stages of activities to maximize the benefits to research for development.

Keywords: banana, Central Africa, East Africa, public-private partnership, tissue culture

Introduction

Cooking banana is a key staple crop throughout East and Central Africa, while dessert banana is an important source of income for many farmers. However, accelerated yield declines of banana have been associated with increasing incidence of soilborne pests and diseases. The use of clean planting material is essential in overcoming such constraints, especially when establishing new fields. Pest infestation or reinfestation, however, remains a vital concern, and therefore the International Institute of Tropical Agriculture (IITA) is researching ways, in collaboration with commercial enterprises, to produce long-term clean banana planting material for the region.

The banana weevil, Cosmopolites sordidus, is the most important insect pest of highland bananas and plantains. Banana weevil larvae tunnel into the rhizome (underground stem), resulting in reduced nutrient uptake, weakening the stability of the plant and causing plants to break. The burrowing nematode, Radopholus similis, is globally the most important nematode pest to banana production and a major constraint in East Africa. Nematodes kill the roots by feeding on them. The destruction of root tissues reduces yield, and ultimately destroys plant anchorage, resulting in a typical symptom of toppling of the whole plant. As with the banana weevil, nematode damage is initially low but gradually builds up over crop cycles. This is particularly disastrous for a perennial crop such as banana: accumulation of pests and diseases results in shortage of adequate, clean planting material.

Banana in the region is traditionally propagated by means of suckers, which are rhizomes cut off from the motherplant. These suckers are infested with soilborne pests such as the banana weevil and the burrowing nematode. Consequently, the use of suckers as a primary means of banana propagation decreases yield and plantation longevity, with dire consequences for food security in the region. In the laboratory, banana can now be produced axenically through tissue culture. A part from viruses, tissue culture banana plantlets are by definition a pest- and disease-free planting material. This banana propagating technique is widely adopted in the world, but is not widespread in East and Central Africa, to a large extent due to the subsistence nature of banana production.

Tissue culture technology offers many other advantages besides being pest- and disease-free. Compared to conventional planting material, tissue culture plants give higher yield, and earlier and more vigorous sucker production. Tissue culture plants are uniform, allow for mass production in relatively short periods of time, and are available all-year round: important criteria for commercial farming. Rapid and easy mass production also allows for facilitated distribution of improved cultivars, and can compensate for planting material shortages.

However, during the early transplanting stages, banana tissue culture plantlets need higher levels of care and attention than conventional planting material. Research at IITA has demonstrated that in Uganda, where soils

Farmer co-opted nursery in Kibirigwi, Mount Kenya region, built with the support of Jomo Kenyatta University of Agriculture and Technology.

are depleted and pests and diseases abundant, tissue culture is only superior to conventional planting material if associated with high levels of field maintenance (1). In East and Central Africa, where banana management practices are often suboptimal and banana production is plagued by constraints, the benefits of a sustainable tissue culture banana system could be huge, and especially so if plantlets could be protected against pests such as the banana weevil and the burrowing nematode.

1 Endophytes: Extending the benefits of clean planting material

Almost all plants are naturally associated with endophytes. An endophyte is an organism that, at some time during its lifecycle, lives within plant tissues, yet does not cause any disease symptoms to its host (2). This association is often mutualistic. Foremost, endophytes provide the plant with antagonism against pests and diseases. Mechanisms by which endophytes protect plants are only recently beginning to be understood, and are manifold. Recent interest has focused on the induction of resistance by endophyte infection of the plant against pests and diseases. Induced resistance is the activation of biochemical and structural plant defence mechanisms following contact with elicitors, such as endophytes (3, 4, 5). Induced plant resistance, elicited by an endophyte, provides a susceptible plant cultivar with pest or disease resistance. 'Immunization' of plants with endophytes therefore can help complement current breeding programs. Endophytes also confer other benefits to the plant. Plant growth seems to be promoted by all major endophytic groups, either through facilitation of increased nutrient uptake (6) or through synthesis of plant hormones (7).

Natural entry of an endophyte into a plant is a process that can be manipulated. Once inside the plant, an endophyte occupies a niche with relatively low competition from other microorganisms, provided the endophyte gets there first. Early entry is key, and can be effected through inoculation during propagation of the plant material. In other words, the endophyte becomes an intrinsic component of the planting material when sold to growers. Reintroduction of endophytes into banana tissue culture plants restores the natural equilibrium and extends the benefits of clean planting material.

2 Circumventing the biopesticide hurdles

Biological control using microorganisms has widely been promoted as an alternative to the use of chemical pesticides. Despite a large body of both theoretical and applied research on the topic, the use of biologically beneficial organisms has been limited, mainly because the same paradigms used for conventional pesticides are being applied to microbial pesticides. Using endophytes as microbial control agents might circumvent some of the problems associated with these paradigms.

Many promising microbial control organisms demonstrate excellent performance in the laboratory against pests or diseases. However, this often translates in below-expected performance in the field because they have to compete with the native flora. This competition can be avoided by using endophytes that escape the rhizospere community where competition is fierce.

Some pests and diseases, such as the banana weevil and the burrowing nematode, are embedded within plant tissues and are therefore not easily controlled. Endophytes offer the potential to control these cryptic pests and diseases.

At practical application rates, the use of microbial pesticides tends to be slow-acting, erratic and expensive. Endophytes can be applied as an intrinsic component of the planting material. This tactic allows for targeted control at low initial inoculation levels, improving consistency of endophyte performance and reducing costs. Added benefits of using endophytes that induce resistance are that 1) endophytic inoculum does not need to be present at the time when the plants are attacked by pests and diseases, and that 2) endophytic inoculum can have a similar effect even at very low doses.

Most importantly, tissue culture plants can be made available to farmers as a 'ready-armed' or endophyte-enhanced plant, removing any need for farmers to apply additional products. Costs and know-how associated with formulation, distribution, application and storage are transferred to a commercial laboratory. Expertise and equipment related to applied microbiology is similar to that of a commercial tissue culture laboratory.

3 IITA's research into banana endophytes

IITA has a mandate to develop sustainable food production systems in tropical Africa, and is linked in the worldwide network of agricultural research centres supported by the Consultative Group on International Agricultural Research (CGIAR). At IITA, research into endophyte-enhanced banana tissue culture was initiated in 1997 under funding by the German Federal Ministry for Economic Cooperation and Development (BMZ). In 2001, BMZ provided substantial funds for a project entitled "Managing micro-organisms to enhance plant health for sustainable banana production in East Africa". This project, which is now entirely focused on the use of endophytes in banana tissue culture, received second phase funding until December 2007 to finalise research needs of this technology. Thereafter, IITA hopes to take the technology into an up-scaling and production phase. Research into endophyte-enhanced banana tissue culture has so far yielded a wealth of information. The protocol used during the research is depicted in Fig. 1 (adopted from 8).

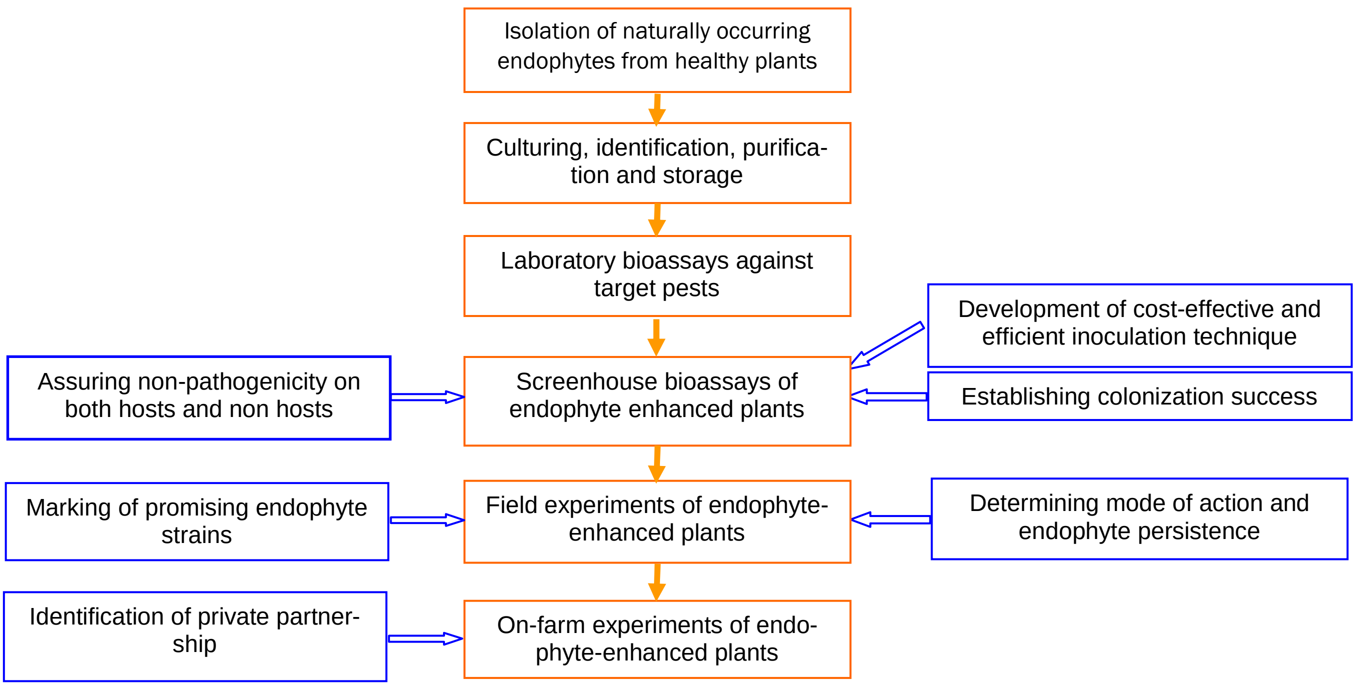
The most critical step in the quest for endophytes is accurate isolation. Since the aim is to find strains that will target specific pests and diseases, IITA's approach is to isolate endophytes from apparently healthy plants growing among high levels of pests or diseases. In addition, the endophytic flora varies with plant cultivar, host plant age and ecological conditions. To isolate endophytes, plant samples are surface-sterilized and plated onto specialized culture media in the laboratory. After identification and purification, they are stored. Following isolations in Kenya, Uganda and South Africa, the most frequently isolated endophytes belong to the genus Fusarium. IITA has therefore focused primarily on this genus.

Based on morphological and molecular analyses, sampling a handful of plants yields a vast array of endophytic strains. Hence, a rapid, easy and cheap laboratory screening protocol was devised to test as many strains as possible against both the banana weevil and the burrowing nematode. Only a handful of strains that show high antagonism against the target pests are further tested. Merging concurrent research into the development of efficient inoculation techniques that yield high colonization, banana tissue culture plantlets are inoculated with these best endophytic strains for screenhouse assessment against the target pests. At present, IITA's best strains are undergoing field testing against both pests.

This logical flow of biopesticide research has been carried out hand-in-hand with upstream research among the partners in the project. Foremost, much effort has been focused towards determination of endophytic modes of action. Fusarium spp. endophytes were found to induce systemic resistance. In addition, many endophytes are individual strains of F. oxysporum, which are ubiquitous soil organisms but can also be destructive wilt pathogens. It has therefore been essential to establish early the non-pathogenic nature of IITA's promising strains, and determine markers to identify them.

The project is comprised of seven partners in five countries: the University of Bonn, Germany; the National Agricultural Research Organization (NARO), Uganda; the University of Pretoria, South Africa; Makerere University, Uganda; Wageningen University, the Netherlands; the Catholic University of Leuven, Belgium; and the Biologische Bundesanstalt fűr Land- und Forstwirtschaft, Germany. Together with IITA, they have laid the groundwork, and endophyte-enhanced technology is now ready to be tested in farmers' fields. Research is not only confined to banana production in Africa. The International Network for Improvement of Banana and Plantian (INIBAP), using a separate source of funding and in collaboration with our German partners, is currently testing endophyte-enhanced tissue culture with large-scale

Figure 1. Overview of research protocol used at IITA for developing endophyte-enhanced tissue banana culture (Based on Dubois *et al.*, 2006).



banana producers in Costa Rica, using Latin American endophyte strains.

4 Banana tissue culture: a constraint to iita's research agenda

One of the greatest uses for endophytes is their application to tissue culture material. The acceptability of tissue culture therefore is critical to the success of this particular work. In large scale commercial banana systems, tissue culture is fast becoming the norm. However, in subsistence agricultural systems in Africa, it is less common, but developing.

IITA does not possess a sufficient in-house tissue culture production facility to accommodate its endophyte-based research. IITA therefore has developed partnerships with key players involved in tissue culture production: the commercial tissue culture laboratory Agro-Genetic Technologies Ltd (AGT), with strong support from NARO in Uganda, and Jomo Kenyatta University of Agriculture and Technology (JKUAT) in Kenya. At first, both public-private partnerships were initiated purely based on a need for tissue culture plantlets as research material. However, these partnerships have proven to be pivotal in steering endophyte-enhanced technology towards the ultimate client: the farmer.

4.1 Agro-genetic technologies ltd, the first commercial tissue culture producer in Uganda

AGT is a privately held Ugandan company that started its operation in 2002. It is owned by Ugandan professionals with several years of experience in agricultural research and entrepreneurship. AGT is currently the sole private commercial tissue culture provider in Uganda. In addition, AGT offers an agronomic consultancy service to its customers. AGT first targeted coffee but is now mainly geared towards banana. In November 2005, AGT opened a new tissue culture laboratory with a production capacity of 8 million plantlets per year, potentially the largest tissue culture laboratory in East and Central Africa.

At present, AGT's main source of sales is through non-governmental organizations and institutions, such as APEP, VEDCO, Caritas, BUCADEF and NARO. However, direct marketing channels with farmers are sought, by conducting seminars in local farming communities, participating in local exhibitions, distributing brochures, and publicity on the radio. To bring the technology near to farmers, AGT has established nurseries and demonstration gardens in 11 locations in Uganda, which act as sales and training centers, respectively. At the nurseries, sales are facilitated through establishment of direct contacts with individual farmers or farmer groups. They are deemed essential to AGT's future business plan. Farmers' nurseries make tissue culture plantlets available directly to the farmers, prevent farmers from sourcing planting materials from neighboring farms and, by doing so, reduce the spread of pests and diseases. They also allow farmers to acquire the desired cultivars in any numbers and at any time of their choice, reduce transportation costs of the ready-to-plant material, and avoid transportation of soil from place to place since the tissue culture plantlets are delivered to the nurseries in vitro. Using farmers' nurseries also facilitated some indirect spill-over effects, such as knowledge distribution to farmers about modern agricultural practices, job creation through recruitment of nursery operators, and fulfillment of the Uganda Government's policy and ambition of modernizing agriculture.

Endophyte-enhanced banana tissue culture plant produced in the nursery of Agro-Genetic Technologies Ltd,

Furthermore, from the outset, AGT has fostered an approach that aims for sustainable social-economical and agricultural development for Ugandan farmers, because AGT is an innovator and needs to create its own market. Areas of innovation include joint exhibitions with research organizations (including NARO and IITA), tissue culture protocol development and student internships with universities (Makerere University), and participation in national biotechnology and biosafety policies (Ugandan Government).

A major constraint of AGT is absence of awareness and distribution channels. This problem creates a vicious circle, as lack of sufficient sales inherently renders tissue culture plantlets expensive due to lack of economics of scale. Other constrains faced by AGT are lack of protocols and equipment.

4.2 Jomo Kenyatta University of Science and Technology: Research with a commercial twist

The Kenyan situation is different from that in neighbouring Uganda, partly as a result of the greater scarcity of planting material, which drove the use and then demand of tissue culture material. Tissue culture technology took off in Kenya, also because of strong impetus from JKUAT. JKUAT has succeeded in transferring banana tissue culture technology to small-scale farmers in Kenya and now produces, on a commercial scale, close to half a million tissue culture plants per annum based on a highly efficient network of farmer-coopted nurseries that it helped develop (Fig. 2).

In 1991, JKUAT started developing banana tissue culture protocols in Kenya. Reflecting the case with AGT, JKUAT quickly realized that the lack of access to, and familiarity with this technology was a key hurdle to small-scale farmer adoption. JKUAT, however, attracted funding to investigate channels to disseminate the technology.

With support from the Rockefeller Foundation, JKUAT facilitated distribution systems that connected the JKUAT laboratory with small-scale farmers in the Mount Kenya region of Kenya. Using a participatory approach, JKUAT engaged in impact studies that enabled an in-depth understanding of the local farming systems, the environment in which the farmers were operating, and, most importantly, a community action plan for adoption of tissue culture. At the onset of the project, farmers were eager for change, due to falling yields and incomes. Coffee had been replaced by banana, but maize and beans still constituted the main staple food crops.

The quality of bananas grown in the area was poor and many farmers grew local varieties that could not be marketed. Pests and diseases were identified as the main problem for banana production in the area. Farmers also realized that, even if they received clean planting material, they lacked the technology and infrastructure for efficient banana production and marketing.

Clearly, these problems could not be solved by provision of banana tissue culture technology alone, so JKUAT embarked on a training programme. At each of the key sites in the Mount Kenya area, JKUAT introduced banana tissue culture nurseries, for operation by farmer groups as private businesses. Simultaneously, training was provided to nursery operators in tissue culture handling and aspects of banana agronomy. Nursery operators trained their own costumers. Development of an own action plan for each of the nursery communities gave them a sense of ownership of the project. The nursery operators receive acclimatized tissue culture seedlings of approx. 10 cm tall, priced at 40 Kenyan Shillings (72 Ksh = 1 USD). Nursery operators grow the seedlings on to field size (90 cm tall), which are marketable at 80 Ksh. The lack of capital and weak marketing channels were addressed through provision of microfinance.

Based on follow-up impact studies in 2004 and 2005, JKUAT realized adoption levels of over 86% at its pilot sites and effectively connected its banana tissue culture laboratory to village nurseries. Since then, its early adaptors have reaped dividends and a substantial number has switched from subsistence to commercial banana farming. However, since introduction of the technology, follow-up impact studies have revealed an unexpected gender shift from female to male, who predominantly manage cash crops in the region.

Some obstacles remain: one of which is the farmers' identified need for pest and disease control, amongst others. Although banana tissue culture plants are initially pest- and disease-free, JKUAT observed that they easily become infested when planted in pest- and disease-infested soils of the Mount Kenya area.

5 Joint research: Benefits for the public and private sector

AGT has entered into an initial two year agreement with IITA. IITA has complete access to AGT's laboratories to fully integrate endophyte-enhanced tissue culture technology in AGT's tissue culture production line (Fig. 3). IITA also has full access to AGT's nurseries for on-farm testing of endophyte-enhanced material. In addition, plants used for IITA's research purposes are purchased for a much reduced price. AGT receives reciprocal benefits. First, AGT has first-hand exposure to endophyte-enhanced tissue culture technology, including endophytic strains. Since the technology has great potential for impact in East and Central Africa, this exposure allows AGT to rapidly capitalize on its benefits. Secondly, IITA, in collaboration with NARO, has initiated research on farmer acceptance of banana tissue culture in Uganda, using AGT's plants and nurseries. Lessons learnt in Kenya demonstrate that producing tissue culture plants is not enough, and investigating ways to mobilize farmers, mainly through nurseries, are essential for success. Finally, AGT benefits through exposure to IITA's scientific world, ranging from meetings with IITA's stakeholders to joint orders for hard-to-obtain chemicals.

IITA's different approach at JKUAT reflects the difference in circumstances with AGT and Uganda. IITA sought a partner in Kenya who could provide experienced feedback towards developing an endophyte-enhanced tissue culture system. JKUAT has proven to be the ideal candidate. As a research institute, JKUAT also contributes towards scientific progress in the field of endophyte-enhanced tissue culture and widen IITA's geographic scope within East Africa. With its commercial tissue culture laboratory, JKUAT can leverage its vast network of farmer-coopted nurseries to rapidly test the technology in the field. Due to quarantine restrictions against Ugandan endophyte strains, JKUAT has engaged in isolation of Kenyan endophytic strains that may also lend themselves better to local agro-ecological conditions. In addition, banana cultivars in Kenya are different to those in Uganda and, as such, JKUAT is equally investigating the potential benefits of endophytes in dessert bananas.

6 Public-private partnerships: yielding unexpected synergisms

In addition to the commercial laboratories providing a vehicle for tissue culture plants, the collaboration has driven IITA and the project activities along commercial thinking. Towards achieving the goal of a useful product, this change in forward thinking has been a beneficial exercise, which will hopefully provide farmers with improved, cost-effective products. Such 'fine-tuning' of techniques and adaption to the practical realities is essential to bridging upstream research with downstream application.

A perfect example has been IITA's experimental protocol for endophyte inoculation. Based on extensive research, IITA devised an inoculation technique that seemed optimal: tissue culture seedlings, after they were removed from the tissue culture flasks, were grown in a nutrient solution for an additional month to enhance root biomass. Collaboration with JKUAT and AGT forced IITA to 'think commercial'. IITA quickly abandoned its use of a nutrient solution in favour of fertilizer-amended soil, along the lines of the system used in the commercial nurseries.

Conclusion

IITA's ultimate goal is to develop an endophyte-enhanced tissue culture technology for farmers' use. Because the technology depends on availability of tissue culture and a venue to farmers, IITA opted to engage in public-private partnerships with AGT and JKUAT. Although this seemed precocious at the time in IITA's research agenda, these partnerships have helped greatly to overcome obstacles, and have proven to be essential to achieve the project's goals. Initially, memorandums of understanding had been carefully prepared, but, after timid beginnings, trust has been established and the momentum has been driven by mutual trust and appreciation, both for each others contribution and the projects' ultimate goal – more durable tissue culture plants, which can be delivered simply and effectively to farmers for improved banana production.

Based on IITA's experience, public-private partnerships should be included at a very early stage. Research for development is impossible without facilitating technology transfer to small-scale farmers from the outset.

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References

1. Niere, B. Unpublished data.
2. Petrini, O. (1991) “Fungal endophytes of tree leaves” In Andrews, J. and Hirano, S. (Eds.) Microbial Ecology of Leaves. Springer-Verlag, New York City.
3. Kloepper, J. W., Tuzun, S. and Kuć, J. A. (1992) “Proposed definitions related to induced diseases resistance” Biocontrol Science and Technology, 2, 349-351.
4. Sticher, L., Mauch-Mani, B. and Metraux, J. P. (1997) “Systemic acquired resistance” Annual Review of Phytopathology, 35, 235-270.
5. Benhamou, N. and Garand, C. (2001) “Cytological analysis of defense-related mechanisms induced in pea root tissue in response to colonization by non-pathogenic Fusarium oxysporum Fo47” Phytopathology, 91, 730-740.
6. Ramamoorthy, V., Raguchander, T., Samiyappan, R. (2002) “Induction of defense-related proteins in tomato roots treated with Pseudomonas fluorescens Pf1 and Fusarium oxysporum f.sp. lycopersici” Plant and Soil, 239, 55-68.
7. De Battista J. P., Bacon, C. W., Severson, R., Plattner, R. D. and Bouton, J. H. (1990) “Indole acetic acid production by the fungal endophyte of tall fescue” Agronomy Journal, 82, 878-880.
8. Dubois, T., Gold, C. S., Paparu, P., Athman, S. and Kapindu, S. (2006) “Tissue culture and the in vitro environment. Enhancing plants with endophytes: potential for ornamentals?” In Teixeira Da Silva, J. (Ed.). Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues. 1st Edition. Global Science Books, London.