CHAPTER 2

CASE STUDIES IN THE CROP SECTOR

Evaluation of the field performance of banana plants derived from tissue culture
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CHAPTER 2.1

USE OF TISSUE CULTURE AND MUTATION INDUCTION TO IMPROVE BANANA PRODUCTION FOR SMALLHOLDERS IN SRI LANKA

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INTRODUCTION

Banana is the world's fourth most important food crop after rice, wheat and maize in terms of total value of production. It is a starchy staple food crop, rich in vitamins A, C and B6, as well as an easily produced source of energy. Compared to other staple crops, banana is cheaper to produce year-round in varied environmental conditions, and highly suited to intercropping and mixed farming systems with livestock, as well as an important subsistence food and source of energy for low-income families, providing food security, nutrition and energy for hundreds of millions of people in tropical and subtropical countries. With urbanization expanding, farmers turn to banana as a "cash crop", which may be the only source of income to the rural population. It serves as a food security crop to the rural community in developing countries including Sri Lanka, and plays an important role in poverty alleviation. Banana also has several applications including the production of alcohol, animal feed and starch; it may be used medicinally, exploited as a fibre or as a source of leaves and to make banana bread, banana dry-chips, baby food and in industrial food processing.

Banana is considered a poor man's fruit crop, widely cultivated in Sri Lanka. There are 55 local banana cultivars in Sri Lanka. Banana growers have small farms, scattered over a large area in the country. The most popular banana cultivars of Sri Lanka are: Embul, or Ambul (Mysore type, AAB group), Kolikuttu (AAB), and from the Cavendish subgroup: Anamalu (AAA), Binkehel (Dwarf AAA), Rathambala (AAA) and Ambon, or Amban (AAA). There is a big potential to expand local markets by educating local farmers through improving (i) plant multiplication technologies (lack of good quality planting material is one major constraint), (ii) post-harvest storage and, (iii) transportation. Embul is the most popular cultivar, covering 65 percent of the cultivated land in the Walawe area alone, the largest banana-growing area under irrigation. Embul is hardy with excellent post-harvest quality and yield. Its slightly sub-acid taste meets the average demand in Sri Lanka more than other cultivars. However, it is relatively tall and late in fruiting in comparison to the Cavendish types.

Banana growers require suitable cultivars for mass-scale cultivation, and need to be able to produce high-yield uniform fruits, drought- and salt-tolerant varieties, and early flowering types. High winds are a cause for concern in banana-growing regions as they damage tall banana cultivars. Shorter banana cultivars should therefore be preferred in high windy areas. Banana yield is very much plagued by several diseases including viral diseases (Thomas *et al*., 1994; Thomas and Magnaye, 1996; Ariyaratne and Liyanage, 2002) such as those caused by the banana bunchy top virus (BBTV), banana bract mosaic virus (BBrMV) or cucumber mosaic virus (CMV). The BBTV disease is quite serious and widespread in the Kandy region of Sri Lanka and BBrMV and CMV are also prevalent in other parts of the country. The banana germplasm,

natural and induced diversity, and conservation are equally important for banana improvement. The loss of genetic diversity can have a serious impact on banana cultivation because of lack of new improved cultivated varieties. There is hardly any disease resistant banana variety available to the growers. The primary needs of Sri Lankan banana growers, which could be achieved by tissue culture and mutation induction, are: a) uniform standard planting material, corresponding to the average demand in the market, b) a shorter Embul c) early fruit bearing, and d) slightly longer fingers in a still symmetrical bunch (Hirimburegama, 1996; Laksiri and Hirimburegama, 1999).

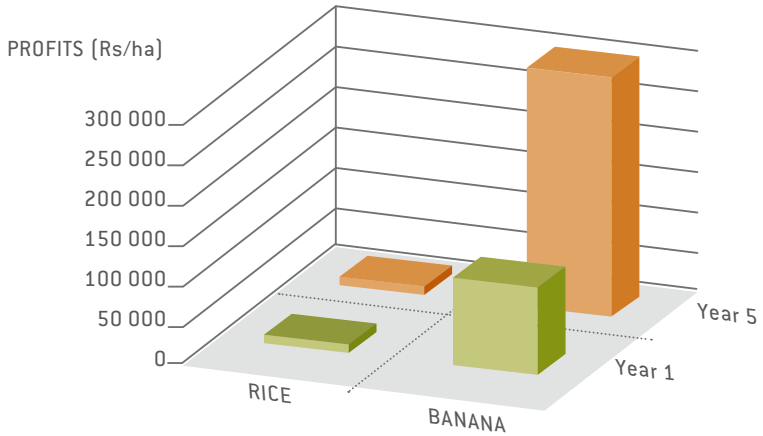
THE PROJECT

The University of Colombo, Sri Lanka began working in collaboration with the Joint FAO/IAEA Division in Vienna, Austria on a banana-growing project. The local authorities, including the Mahawelli Economic Agency, Irrigation Department, Deputy Minister of Agriculture, Export Development Board, Atomic Energy Authority, Southern Development Authority and NGOs came together to support banana projects at the University of Colombo. Extension services were used to educate small farmers for planting micropropagated banana plants and to monitor improvements in their economic status (Rodrigo *et al.*, 2003; Hirimburegama, 2005).

Small farmers make up the majority of banana growers in developing countries, producing for home consumption and local markets. In Sri Lanka, the banana is considered a priority fruit, next to rice. Until recently, banana cultivation was restricted to subsistence farms and backyards but the importance of larger plantings has been recognized. The targets of biotechnology innovation were small farmers with small land holdings from 0.5-2.5 ha, who normally use conventional propagation methods (Naseem and Xiao, 2009). They could increase their profits fivefold by combining land through banana grower associations and cultivating land jointly. Private banana farms can be as large as 10-50 ha. Moreover, the Sri Lankan Government has selected banana as the reference crop in the drive to crop diversification, as well as for large-scale commercial production and to create employment among economically deprived women in the rural sector who are financially dependent on their husbands (Figure 1). With increased problems in rice cultivation, mainly due to insufficient water supply (about half of the total irrigated soil surface cannot get the amount of water required), farmers would like to change from rice to banana cultivation. During the last years, more than 2 500 ha have been converted from rice growing to banana plantations, quite a significant increase. The main reason for this shift in agriculture is high input and low economic returns from rice, and comparatively low input and high income from banana (Figures 1a and 1b).

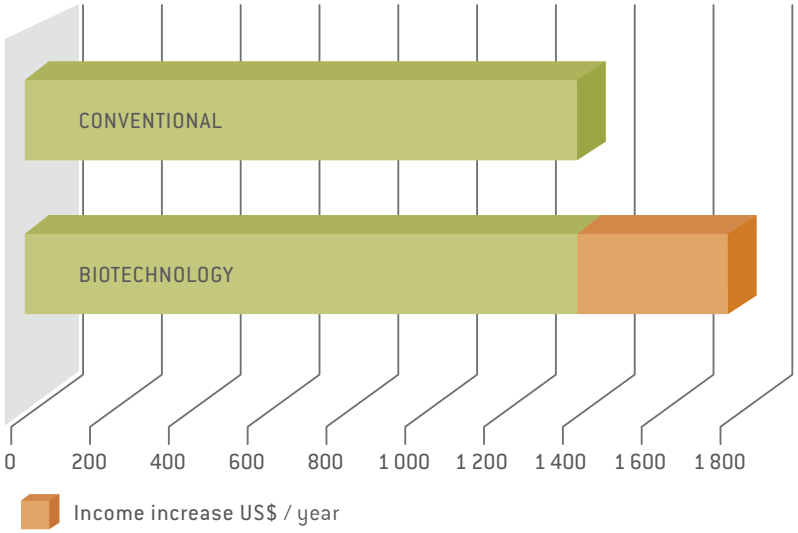
Figure 1. Impact of banana cultivation and impact of biotechnology on banana cultivation value

FIGURE 1a. IMPACT OF BANANA CULTIVATION



Rice growers remained economically backward due to low economic returns on harvest. The average rice grower makes a total profit of 4 500 rupees (Rs.) per ha by spending 6 000 Rs. The first year profit of a banana grower is 84 000 Rs. per ha, and during subsequent 2-5 years, profits can go up to 300 000 Rs. per ha. Due to such a huge profit margin in banana cultivation, growers have shifted from rice to banana cultivation.

FIGURE 1b. IMPACT OF BIOTECHNOLOGY ON BANANA CULTIVATION VALUE



Dwarf micropropagated banana plants have an advantage that they can be grown closely compared to tall varieties by reducing the distance between plants from 3.0 to 2.5 m. Early fruiting and harvesting times of micropropagated banana plants save at least one month. The number of ratoons (second and subsequent crop from the suckers) will be two in two years in micropropagated plants compared to three years in conventionally propagated plants. Thereby, the income of farmers will increase by 25 percent, which is about US$350 per acre per year. As the demand for micropropagated plants will increase, their selling cost will also go up substantially, e.g. from 15 to 20 Rs. per plant.

Source: Hirimburegama, 2005

The Uda Walawe area is the largest banana-growing area especially under irrigation in Sri Lanka, and the University of Colombo developed an Agrotechnology and Community Service Center at Weligatta, Hambantota, which is one of the most remote areas of the country. The purpose of the Center is to transfer the technology to the growers by a farmer's participatory approach. A tissue culture laboratory was set up at the Center where locals are trained to produce plants through the shoot-tip culture technique. Farmers living in this area have a monthly income of less than US$9 (900 rupees [Rs.]). The proposed plan was to grow food crops other than rice. Banana had been identified as one of the main crops for cultivation in this area. The Weligatta Center assists farmers in banana cultivation. As the economic returns of farmers improved, the banana cultivation area increased from 45 000 to 50 000 ha in 1998. Banana has become a cash crop in certain parts of the country. Under future schemes, new areas will be included.

Between 1994 and 2001, the Department of Botany of the University of Colombo was involved in an FAO/IAEA coordinated research programme on "Cellular biology and biotechnology, including mutation techniques for creation of new useful banana genotypes" and in two IAEA technical cooperation projects since then (the last one closing in 2008). So far, they have obtained mutants for earliness and dwarfness by gamma irradiation of Embul shoot tips. Some mutant lines showed slightly higher yield than the parent lines. Since then, the possibility of improving yield by *in vitro* mutagenesis has been continuously explored by Sri Lankan scientists (Hirimburegama and Gamage, 1997; Sirisena and Senanayake, 1997; Hirimburegama *et al.*, 2004).

Mutation induction coupled with selection remains the cleanest and most inexpensive way to create varieties by changing single characters without affecting the overall phenotype. Mutation induction involves the treatment of plant propagules with mutagens (e.g. gamma-rays). This is followed by selection for desirable changes in the resulting mutants. Breeders use mutation induction to broaden the genetic base of germplasm, and use the mutant lines directly as new varieties or as sources of new variation in breeding programmes. Mutation induction assisted plant breeding is a proven, cost-effective and unregulated methodology, as proven by the more than 3 200 officially released mutant varieties of more than 200 plant species in close to 100 countries.

Plant tissue culture advances, including somatic embryogenesis, micropropagation and micrografting, cryopreservation of embryogenic cell cultures, *in vitro* selection of cells and tissues resistant to fungal toxins, somatic hybridization and embryo rescue have already been applied to tropical and subtropical fruits.



from left to right
In vitro plantlets of banana after micropropagation
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Banana plantlets transferred from *in vitro* conditions to potting soil (to slowly adjust to natural conditions)
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Production of banana derived from micropropagation in the farmer's field
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Molecular markers have also been applied for identifying useful germplasm in several crops. The selection of cost-effective technology is highly desirable for sustainable banana improvement, which can be readily financed by the government. Initially, international organizations such as FAO, IAEA, Bioversity International (ex-INIBAP) and the Common Fund for Commodities, among others, assisted the government in setting up the infrastructure, supplying chemicals and equipment, manpower training and expert consultations. So far, Sri Lankans have used tissue culture for large-scale multiplication of local elite Embul. There was, however, a continuously increasing demand for banana planting material, and the local resources were very limited and unable to meet the demand of the banana growers, which forced the government to import planting material from other countries.

The imported planting material takes time to adjust to the new environmental conditions, and imported banana plants became infected with viruses. All imported tissue culture banana planting material is handled by the Quarantine Section of the Department of Agriculture. The

success of micropropagating mutant banana varieties engendered an urgent need to reliably test the banana mutants developed and produced through mutation breeding on a large scale for virus diseases. Virus diseases are serious for banana cultivations and tissue culture mass production is a very easy way to spread the diseases caused by viruses if precautions are not taken during production. The major objectives of producing micropropagated banana plants therefore are: a) to produce virus-free healthy mother stocks, b) to eliminate viruses from promising germplasm, and c) to make promising plant material available to growers. But this process must be monitored for quality control (Naseem and Xiao, 2009).

In Sri Lanka, imported commercial kits were used to test pathogenic viruses of banana as well as all other crops. As the mutant banana locally developed was Embul, imported diagnostic kits were available for only two of the three viruses mentioned earlier (i.e. BBTV, CMV and BBrMV) and were not reliable for local virus isolates. Cost is also a limiting factor. But local capabilities were available to produce adapted local kits and to develop an enzyme-linked immunosorbent assay (ELISA) for the third virus. Viruses were isolated and injected into hens in Sri Lanka by the counterpart, which successfully produced an initial ELISA kit that needed further standardization and validation. The method of using hens (antigens isolated from eggs) was selected to avoid bleeding of animals such as rabbits and rats. In addition to using the ELISA method for the virus diagnosis, a more sensitive polymerase chain reaction (PCR) methodology was also transferred. This is required especially when the virus disease symptoms are not present in a plant that is nonetheless infected. Both technology packages for the three viruses are now available to monitor the production process (Hu *et al.*, 1995; Dassanayake and Rathnabharathi, 2002; Ahloowalia *et al.*, 2004).

In this multidisciplinary project, where technology has been transferred to the rural farmers in a participatory research approach, IAEA technical cooperation projects have provided all equipment and chemicals required for the project as well as for teaching and research purposes of undergraduates and post-graduates and one fellowship to transfer the technology packages. An ELISA reader was obtained from a local NGO. A new building was provided by the University of Colombo for the project work.

Above all, the tissue culture laboratory established in a rural location in Sri Lanka has already transferred the mutation breeding product (a banana mutant). Virus-free banana mutant plants are produced; farmers are being trained, guided to obtain an export quality product in an environmentally friendly manner without spraying pesticides, while using organic matter with minimum amounts of synthetic fertilizers. There is a great demand for tissue cultured banana plants all over the country, and this is the only programme where plants are produced

for rural farmers in Sri Lanka while farmers are guided by the project personnel. The main banana project of the University of Colombo is currently developing another two tissue culture laboratories in the country.

Initial obstacles and challenges had continuously to be addressed, but the growing success strengthened the political will to continue support. A conservative management attitude conflicted with a new approach and vision. By way of example: the initial negative and non-cooperative attitudes of some authorities, groups and individuals at higher levels and their failure to recognize that technology transfer and implementation are tasks for multidisciplinary teams created a negative effect on development activities. In addition, common in developing countries, frequent interruptions of electricity and water supplies, and sociopolitical changes, slowed the project implementation. But the continuous accumulation of successful implementation steps strengthened the next ones and produced substantial results (Table 1).

Table 1. Project impact

OUTPUT	IMPACT	OUTLOOK
More than 15 000 plantlets produced per month	Around 500 farmer families are involved in tissue culture banana cultivation, spreading all over the country, farmers are being organized into clusters	Export on trial basis to Japan
Mutant and other tissue culture virus-free banana plants developed through this project are in high demand by cultivators	Value-added banana chip product has been commercialized by an entrepreneur	Income increase up to 25-fold
University system has established the first agro-technology and community services centre which is a self-financed technology competence centre for the rural sector development in a multidisciplinary setup coupled with education	• R&D status of the University of Colombo has been improved, and the national science policy is being developed through the Sri Lanka National Science Foundation • Ministry of Science and Technology has recognized this project as a model for agrobiotechnology implementation in the country	A small unit of the Ministry for other technologies established at the university centre
Banana growers associations can provide raw material to the industry without middlemen. A program on banana chips, flour and jam was started. This has been introduced in small-scale in several adjoining districts of Sri Lanka	Sustainability for an environment-friendly new developmental approach established with public participation and 'political will'	Start-up of a banana agrifood industry in underdeveloped districts of Sri Lanka

Given the initial challenges, the main factors of success were that the government took ownership and supported the project over the long term at the regional and national level, and that the project involved a variety of stakeholders such as the national agricultural research and extension systems (NARES), farmers' associations, NGOs, the private sector and academia, and enjoyed the continuous support of the Joint FAO/IAEA Division through technical cooperation including technology transfer, capacity development and policy advice.

Biotechnologies have the potential to provide resources for genetic improvement of vegetatively propagated crops such as banana, through mutation induction, screening and propagation techniques, which can now be integrated with conventional techniques. Banana has thus become a "cash crop" in Sri Lanka. The results show that the micropropagated banana can greatly contribute to the exchequer as compared to conventional methods. In the entire country, there is a continuously increasing demand for micropropagated banana plants (in the Hambantota district alone, 20 000 Embul plants per season can easily be sold). The Federal Government is supporting banana plantations and has provided land to the Weligatta tissue culture laboratory. The local Ministry fully supports the programme and provides timely assistance. Women from this rural sector, who had previously stayed at home without any hope for future personal development, are now employed in plant production and enjoy improved livelihoods (they can afford to purchase houses, television sets and other electric appliances).

Cost-effective and labour-oriented biotechnologies (tissue culture, mutation induction) can support rural development by creating job opportunities, opening the job market also to women, providing a scope for use of raw materials for industry and thereby improve food security, nutrition and the economy.

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A happy pearl millet farmer family after they have just harvested a bumper crop
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CHAPTER 2.2

SUCCESSFUL MARKER-ASSISTED SELECTION FOR DISEASE RESISTANCE AND DROUGHT TOLERANCE IN PEARL MILLET IN INDIA

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INTRODUCTION

Pearl millet (*Pennisetum glaucum*) is grown for grain and stover (dry fodder) in some of the hottest and driest areas of Africa and South Asia. It is a crop that is able to produce nourishment from the poorest soils in the driest regions in the hottest climates, where no other cereal can grow. However, not even pearl millet can grow when there is no water, and the low and very unpredictable rainfall of the areas in which it is grown results in extremely unstable yields. The major disease of pearl millet is downy mildew (DM), caused by *Sclerospora graminicola*, and can result in up to an 80 percent yield loss as the grain is replaced by leaf-like structures. Downy mildew is particularly problematic on genetically uniform single-cross pearl millet hybrids, which are grown on over half of the crop's area in South Asia, and prior to about 2000 had overcome the resistance of nearly every pearl millet hybrid that had become popular (and hence widely and repeatedly cultivated by farmers) in India.

Success in improving genetic tolerance to drought and resistance to mildew has traditionally been very slow and difficult. In 1990, the Plant Sciences Research Program of the UK Overseas Development Agency (ODA, now known as the Department for International Development [DFID]), began funding a series of collaborative research projects on pearl millet involving scientists at the Institute of Grassland and Environmental Research (IGER, now part of the Aberystwyth University and known as the Institute of Biological Environmental and Rural Science [IBERS]); the University of Wales, Bangor; the John Innes Centre (JIC); and the International Crops Research Institute for the Semi-Arid tropics (ICRISAT) in India. They were later joined by scientists from several agricultural universities and central government research institutes/programmes in India. The objective of the collaboration was to develop genetic maps based on molecular markers and use them for better understanding and breeding of traits, such as disease resistance and drought tolerance, for the benefit of smallholder pearl millet farmers across Africa and South Asia.

DEVELOPMENT AND USE OF MOLECULAR MARKER TOOLS

The development of pearl millet molecular markers and genetic maps, their use in putative quantitative trait locus (QTL) detection and validation and their successful deployment in the improvement of elite hybrid parental lines via marker-assisted backcrossing (MABC) methods involved the active collaboration of several partners. The collaborative effort first established molecular marker maps in pearl millet (Liu *et al*., 1994) which provided the basis for studying the genetic basis of traits and also the identification of QTLs and associated markers

Scientists discussing genotyping differences in grain filling under drought stress
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(Bidinger *et al*, 2007; Jones *et al*., 1995; Yadav *et al*., 2002, 2004, 2011) for use in marker-assisted breeding. This effort was initially guided by genetic markers called restriction fragment length polymorphism (RFLP) markers but newer and easier to screen markers were taken on board as they were developed (Qi *et al*., 2004; Sehgal *et al*., 2012). Once QTLs were identified and validated, the mapped QTLs were transferred to economically important background using marker-assisted backcrossing (Howarth and Yadav, 2002) to accelerate the process of plant breeding. For example, the conventional backcross transfer of DM resistance to improve the seed parent of the popular hybrid HHB 67 took nearly nine years (1991-1999), while marker-assisted backcross transfer to improve the pollen parent of that hybrid (Hash *et al*., 2006) was completed in just over three years (1997-2000), once the markers had been developed and marker-trait associations established (1990-1995).

The process of identifying parents, making trait-specific crosses, developing mapping populations, creating and mapping RFLP-based genetic markers started in the early 1990s. Crosses were made at ICRISAT along with advancement of the mapping populations. JIC developed the DNA markers; IBERS transferred these onto trait-specific crosses and developed

genetic linkage maps. Downy mildew screening was conducted at the University of Wales, Bangor. QTLs for traits were developed through collaboration of IBERS, Bangor and ICRISAT. Marker-assisted backcrossing was developed by ICRISAT in collaboration with IBERS and applied in the development of 'HHB 67 improved' through the involvement of the Chaudhary Charan Singh Haryana Agricultural University (CCSHAU), Hisar in India.

Pearl millet breeding teams in India at ICRISAT and CCSHAU used the technology to introgress two major QTLs for downy mildew resistance from the donor parent ICMP 451-P6 into the genetic background of the elite male parent H 77/833-2, the male parent of three released hybrids (HHB 60, HHB 68 and the very popular extra-early maturing hybrid HHB 67) that had been developed at CCSHAU and released for cultivation in Haryana State, and later in all of India. From nine "improved" versions of the pollinator, two were selected for wider testing, along with the original pollinator, in a line x tester study during the 2001 rainy season, and four improved versions of HHB 67 were selected for inclusion in national trials. Two of these were advanced through the first year of national trials and were advanced for another two years testing in trials targeting the shortest growing season areas where pearl millet is cultivated in India. At the same time, extensive on-farm trials of the two improved versions of HHB 67 and the original hybrid were conducted in Haryana State.

On the basis of on-station trials at the state and national level, and on-farm testing at the state level, the taller and slightly later flowering of the two improved versions (preferred by farmers because of its higher stover yield) was selected for release in 2005 as a more downy mildew resistant and higher yielding replacement for the original HHB 67, and named "HHB 67 Improved" (Hash *et al*., 2006).

FUNDING

From the time that marker development was initiated until HHB 67 Improved was released, a series of research grants from DFID's Plant Sciences Research Program funded marker development, linkage map construction, QTL detection, and marker-assisted backcross transfer of identified QTLs to hybrid parental lines for disease resistance, drought tolerance and other traits. In addition, substantial support was provided from ICRISAT core research funding (through the Consultative Group on International Agricultural Research) and, during the hybrid testing phase, there was considerable state and national support for the CCSHAU pearl millet breeding section and national support for the All-India Coordinated Pearl Millet Improvement Project (AICPMIP). Following the release of HHB67 Improved, there was substantial breeder seed

multiplication by both ICRISAT and CCSHAU, and this seed was distributed to both public sector and private sector seed companies. Apart from DFID, there has recently been additional funding (2008-2012) from DFID and the Biotechnology and Biological Sciences Research Council under the Sustainable Agricultural Research for International Development initiative targeting improved drought tolerance.

MILESTONES IN PRODUCT DEVELOPMENT AND DISSEMINATION

The use of marker-assisted selection (MAS) to improve a locally adapted pearl millet variety and the dissemination of the new and superior variety required the collaboration of several institutions, scientists and funding agencies across two continents (Asia and Europe). The sustained commitments of these partners, from tools development to the release of the new variety, has spanned over twenty years (1989 to 2010). The highlights of this collaborative endeavour include:

- 1989: HHB 67 pearl millet hybrid released at the state level in Haryana. This hybrid was the earliest maturing pearl millet hybrid ever released and was rapidly adopted over the subsequent five years by dryland farmers in Haryana and neighbouring Rajasthan.
- 1990-1995 (and beyond): Creating and mapping RFLP-based markers for pearl millet, then identifying marker-trait associations for resistance to DM, drought tolerance and other traits.
- 1991-1999: Conventional backcrossing of downy mildew resistance from ICML 22 into the background of seed parent maintainer line 843B by ICRISAT.
- 1995-2000: Marker-assisted backcrossing to improve parents of HHB 67 and initial hybrid testing (by ICRISAT).
- 2001: Initial target environment assessment of "improved" hybrids in Haryana and Rajasthan (by ICRISAT, CCSHAU, Rajasthan Agricultural University and AICPMIP).
- 2002: Three years of on-station trial evaluation initiated at state (CCSHAU) and national (AICPMIP) levels (required for governmental approval).
- 2002-2004: Three years on-farm evaluation with Haryana farmers (collaborative effort of ICRISAT and CCSHAU).
- 2003: Promotion of the two improved versions of HHB 67, by the AICPMIP (national programme) coordinator, into national trials targeting the driest production zone (where they were yield-competitive and could complete the necessary second and third years of national testing required prior to release proposal development).
- 2005: HHB 67 Improved released by the Indian Government for commercial cultivation as a higher yielding and more downy mildew-resistant alternative to the original HHB 67, following its proposal at state and national level by the CCSHAU pearl millet breeding team (with support from AICPMIP and ICRISAT).

- 2005: Initial production of Breeder Seed of the parents of HHB 67 Improved by ICRISAT in anticipation of its release for commercial multiplication and cultivation.
- 2006: Commercial hybrid seed multiplication for 30 000 hectares and initial marketing in Haryana and Rajasthan; outbreaks of DM on the original HHB 67 in Haryana stimulate farmer interest in its replacement, which had been delivered "just in time"; Breeder Seed of parents of HHB 67 Improved given (free of charge) to companies requesting parental lines of the original.
- 2007-2009: Commercial hybrid seed multiplication increases to permit sowing of 60 000 to 100 000 hectares in 2007 to more than 500 000 hectares in 2009; Breeder Seed of the parental lines is now sold to the concerned companies; and spread of the downy mildew outbreak on the original HHB 67 forces its replacement in Haryana.
- 2010: Hybrid seed production sufficient to sow up to 700 000 hectares for the 2010 rainy season (circa 350 000 farm families).

IMPACTS

With the official release of HHB 67 Improved in 2005, the economically useful lifespan of the earliest maturing pearl millet hybrid available to farmers in India has been extended. This hybrid was rapidly adopted by farmers (particularly those farming under dryland conditions) first in Haryana and later in parts of central and western Rajasthan, where its extra-early maturity reduced crop vulnerability to terminal drought stress and made it possible to extend the area where rainfed double cropping could be practised. The adopting farm families, and the seed industry that supplied them with hybrid seed, were thus able to continue with the cropping system that HHB 67 had permitted. HHB 67 Improved is now massively grown on farmers' fields in India (ICRISAT 2012). Other than for its improved yield and DM resistance, HHB 67 Improved fits into the production niche originally occupied by HHB 67, which has aided its rapid spread and high adoption rate. From an initial 80 tons of unregistered seed that was distributed by the Department of Agriculture and Cooperation (DAC) of the Government of India in 2005/06, seed production of HHB 67 Improved increased continuously, reaching 3 491 tons in 2010/11 (ICRISAT, 2012) when over 1.1 million packets of seed were distributed to farmers. This demonstrates its rapid rate of adoption by the Indian seed industry and pearl millet-producing farmers in north-western India. In comparison, seed production for HHB67 rose from an initial 81 tons in 1991 to a peak production of 2 835 tons in 1999. By 2008, it was phased out from the production chain because of its increased susceptibility to DM (Jones *et al*., 1995).

ICRISAT (2012) summarized the impacts of HHB 67 Improved as follows: At the peak of its adoption, HHB 67 was cultivated on about 774 000 ha in Haryana and Rajasthan, while the cultivation of HHB 67 Improved rapidly spread to 875 000 ha by 2011 (six years after its release in 2005). The net additional benefits to the Indian farming community from cultivation of HHB

67 Improved over the local landrace varieties in Rajasthan and over HHB 67 in Haryana in 2011 alone reached Rs 675 million (US$13.5 million). On average, seed production of HHB 67 Improved generated Rs 65 679 (US$1 314) per hectare of net income to seed producers (primarily in Andhra Pradesh and Gujarat), with a total net benefit of Rs 318 million (US$6.4 million) in 2011 alone. Hybrid seed multiplication also generated 186 person days of employment per hectare (10 times more than grain production), resulting in a total of 900 000 person days of employment, of which 45 percent were of women labourers. HHB 67 Improved also helped stabilize pearl millet production. Its higher yield released land for crop diversification allowing the cultivation of cash crops such as sesame, cluster bean and food legumes. Further, the short duration of both HHB 67 and HHB 67 Improved facilitated the cultivation of winter season rotational crops such as mustard, wheat and chickpea, thus doubling cropping intensity and substantially increasing income compared to previously grown pearl millet landraces (ICRISAT, 2012).

In conclusion, this technologically advanced research has brought greater food security to around 2 million people, who grew the previously popular but DM susceptible variety HHB 67 and whose crops were previously at risk from DM. The new pearl millet hybrid, HHB 67 Improved, is grown in northern India (primarily in the states of Haryana and Rajasthan) by resource-poor farmers in areas where frequent droughts substantially reduce yields, and is able to escape end-of-season drought because it is very early to mature and is resistant to downy mildew, the most devastating disease of pearl millet.

On the negative side, the area sown to HHB 67-like hybrids has expanded even further, making the production system more vulnerable in the short term. However, continued maintenance breeding to enhance the downy mildew resistance of the hybrid's parental materials, backed up by seed dressings that reduce the likelihood of downy mildew infection during early seedling growth, should keep downy mildew at bay for some time to come.

LESSONS LEARNED

As the justification for funding much of plant molecular biology research is improvement of breeding programme effectiveness, interesting molecular biology and publications are not enough to achieve success in development of marker-assisted breeding tools – provided that success is defined as adoption of products of the technology by consumers at the farm level. Strong linkages (that are adequately financed) with applied breeding, varietal evaluation and the seed industry – as well as managers all along the technology delivery pipeline – are required for success, as well as the "biotechnology".

The overall success of this multi-stakeholder endeavour may be ascribed to a number of critical factors that include:

- Focus on a critical constraint to production of the crop (downy mildew disease), for which appropriate sources of resistance and phenotyping methodologies were well known but cumbersome to use effectively in applied breeding, and in which many breeding programmes and seed companies were interested; and with the economic importance of the constraint well understood by breeders, pathologists, seed companies and research managers.
- Long-term support from the donor (16 years from 1990 to 2005), with critically guided movement towards application at the earliest reasonable opportunity – well before the availability of "breeder-friendly" markers – and at the expense of more rapid development of breeder-friendly markers, with agreement to this by the biotechnologists in the team.
- Involvement of the end users (the CCSHAU millet breeding team) in the MAS programme for the pollinator (undertaken at ICRISAT by a PhD student co-guided by staff from CCSHAU and ICRISAT), and focus on improvement of "their best baby" (i.e. HHB 67), even before it began to show indications of disease susceptibility in the target environment, greatly facilitated inclusion of the experimental improved hybrids in state and national trials.
- Early involvement of the management of the Indian national programme, while starting the movement towards application, so that when opportunities to assist moving the biotechnology product forward in the national testing programme occurred, they were dealt with in a fair way that was favourable to said products.
- Information campaign to make seed producers aware of the vulnerability of the original hybrid, and of the ready availability of Breeder Seed of the parental lines of the improved version resulted in a transition that was so smooth that many farmers now growing the improved version do not recognize it as being different from the original.
- Widespread sharing of information with those who could use it, prior to publication.
- Co-authorship extended, as appropriate, to all actively involved in each stage of the process.
- JIC waived its intellectual property rights to the RFLP markers thereby enabling the freedom to operate necessary for the deployment of these tools in the breeding of pearl millet for smallholder farmers in developing countries.
- CCSHAU made the pollinator of its popular hybrid HHB 67 available for use as recurrent parent, and then took responsibility for selecting two improved versions of that hybrid for more extensive evaluation in state and national trials, and ultimately proposed one of these for release.
- The seed parents of HHB 67 (843A and 843B) were developed at ICRISAT from materials introduced from Kansas State University, and numerous improved versions of these were then developed by ICRISAT by both conventional and marker-assisted backcrossing.

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Researchers, extensionists and farmers interacting on a farmer's field with plantain and banana hybrids intercropped with cocoa in the Assin District

CHAPTER 2.3

CLEAN PLANTING MATERIALS PRODUCED *IN VITRO* TO IMPROVE PERFORMANCE OF SWEET POTATO, PLANTAIN AND BANANAS IN GHANA

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INTRODUCTION

Ghana is a developing country in West Africa working towards the consolidation of its recent reclassification as a middle-income economy. The Council for Scientific and Industrial Research-Crops Research Institute (CSIR-CRI) is the lead national agricultural research organization whose mandate is to conduct research to serve the resource-poor farmers in Ghana. For crop improvement, biotechnology tools that enhance efficiencies are being used to complement conventional breeding methods. CSIR-CRI initiated the integration of biotechnological tools in its research and development (R&D) activities in 1996. Before that, the lack of skilled human resources (Quain *et al*., 2012) made such a step unfeasible. The biotechnology R&D facilities of CSIR-CRI include tissue culture and molecular biology laboratories.

Plant tissue culture refers to the set of techniques employed in maintaining or growing plant cells, tissues or organs under sterile conditions on nutrient culture media, usually of precisely determined composition. This and its associated techniques can be used for the generation of disease-free (clean) planting materials, the rapid multiplication of plant propagules and the conservation of germplasm. Biotechnology tools developed at CSIR-CRI have contributed immensely to the development, evaluation, release and dissemination of crop varieties in Ghana.

PROBLEMS REQUIRING BIOTECHNOLOGY TOOLS

It is evident that over the past few decades, agricultural productivity has increased significantly throughout the world. Nevertheless, such increases have not been recorded in a significant part of Africa with the result that smallholder farmers, particularly in the sub-Saharan region, continue to struggle to grow enough food to feed and care for their families. One reason for this is that the smallholder farming systems are hardly ever provided with reliable access to high-quality seeds and planting materials, in spite of the demonstrated contribution of these critical inputs to increasing agricultural productivity.

Unlike grain and cereal crops which are propagated by seed (where thousands of seeds can be generated in a single harvest), vegetatively propagated crops have low multiplication rates. Diseases are devastating because they are also passed down systemically from generation to generation being endemic in the planting material. The consequence is a reduction in crop yields, which, for plantains and banana, can be about 40 percent in the first year of production.

The movement of vegetative planting materials leads to the dissemination of associated diseases. In crops such as yam, cocoyam, taro and sweet potato, the edible part serves as the

planting material. In others, such as plantain, banana and cassava, non-edible parts are used. With these crops, it is very difficult for farmers to purchase and transport sufficient quantities of planting materials. Nonetheless, these clonally propagated crops have the potential to increase agricultural productivity if the quality (physiological, genetic and sanitary) of their planting materials is improved. It is therefore paramount that tissue culture be utilized to rapidly produce clean planting materials for dissemination to crop-growing regions. In this case study, we focus on sweet potato, plantain and banana in Ghana.

Sweet potato is one of the staple crops in Ghana. Food and Agriculture Organization (FAO) data show that until 1996 there were no official figures for the production of sweet potato in the country (FAOSTAT, 2011). Consumption of the tuber was for a long time limited to its use as a snack during the peak season of its harvest. This can be attributed to the availability of other root and tuber crops (yam, cassava and cocoyam), which are very popular in the Ghanaian diet. In the early 1990s, research efforts in Ghana sought to enhance the acceptability of sweet potato for consumers. One of the setbacks encountered was the lack of availability of planting materials. This is because the conventional mode of propagating sweet potato is asexual, being done through the vegetative vine or edible tuber. Accumulation of the inoculum of pathogens (viruses, bacteria and fungi) in these propagules over time results in poor quality planting materials with severely reduced yields.

In 1998, four sweet potato varieties were released in Ghana by the National Varietal Release Committee. The varieties were called Faara, Sauti, Okumkom and Santum Pona (Otoo *et al*., 1998). As the released varieties might have accumulated pathogen inoculum over the years of field trials, the Varietal Release Committee prescribed that the released varieties of all vegetatively propagated crops be cleaned and virus indexed before multiplication and eventual introduction into the seed systems for distribution to farmers. The value of cleaning and indexing sweet potato plants had never been demonstrated in Ghana, though it was known that these practices can increase yield by up to 40 percent. This project targeted smallholder farmers at Okyereko and its environs in the Coastal Savannah agro-ecological zone of Ghana from 2000 to 2003. Funding for the production of clean sweet potato planting material using tissue culture methods was provided by the International Fund for Agricultural Development (IFAD), as Ghana benefited from the Root and Tuber Improvement Program between 1999 and 2004 (IFAD, 2004).

Plantains and bananas are important starchy staples in Ghana. They are also of great socio-economic importance in the country and are very important food security crops in the marginal coastal zones of the country, both as energy-yielding staples and sources of micronutrients, provitamin A and other minerals. In Ghana, plantain consumption is estimated at approximately

84.4 kg/cap/year (MOFA-SRID, 2012). The main limitation to production has been the availability of clean planting materials. The first biotechnologically (embryo rescue in tissue culture) developed plantain and banana planting materials to be used in Ghana were from the Fundacion Hundurena de Investigacion Agricola (FHIA) (Dzomeku *et al*., 2007a) and included FHIA-01, FHIA-03 and FHIA-21. Funding for the evaluation of these materials in Ghana was provided by the International Development Research Centre (IDRC), Canada. Evaluation of these new materials led to the release of one plantain and one banana variety in Ghana in 1999. During the field evaluation, the CSIR-CRI tissue culture laboratory rapidly produced the required quantities of clean and healthy planting materials for smallholders.

Also, plantain and banana hybrids developed at the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria, were evaluated in Ghana. Again, the CSIR-CRI tissue culture laboratory produced healthy seedlings for evaluation on smallholder farmers' fields in six regions of Ghana. This study was supported by the Gatsby Charitable Foundation of the United Kingdom (Dzomeku *et. al*., 2007b). The Ghana Government funded the improvement of biotechnology techniques in CSIR from 2006 to 2008 and this paved the way for the optimization of the protocols for the rapid multiplication of local clones of plantain and banana (Quain *et al*., 2010). The United States Agency for International Development (USAID) also supported the evaluation of selected hybrid plantains and bananas for three years in the Assin districts in the Central Region of Ghana. The collaborative project was implemented jointly by CSIR-CRI, World Vision Ghana, Ministry of Food and Agriculture, IITA, Bioversity International and farmers (Dzomeku *et al*., 2008b). This funding supported 500 farmers to evaluate new hybrid plantains and bananas in the first year. In the second year, an additional 500 farmers were included in the project and supplied with planting materials from the first group of farmers. Tissue culture seedlings were distributed to farmers in the first year and on-farm macro-propagation techniques were used to multiply planting materials in the second year.

BIOTECHNOLOGY TOOLS USED

It is known that the cells within the shoot tip of a plant multiply faster than the viruses, bacteria and fungi in the plants. Consequently, if one is able to isolate cells at the shoot tip and grow them on appropriate medium under sterile conditions, the plants that are generated are free of pathogens. Plants generated through tissue culture are thus "clean". Tissue culture techniques are capable of rapidly multiplying plants vegetatively under sterile conditions at a faster rate than by conventional propagation methods. Tissue culture is not limited by the weather, so production can be done all year round in a limited space, and the system can also be used to conserve plants for posterity.

The technique of excising shoot apical cells (meristems) which are apparently free from fungal, bacterial and viral infections for growth in culture media for the production of clean planting material was used in this case. An electron microscope was used to observe sap from the plants to ascertain that the plants were free from viruses. Micropropagation was used to rapidly multiply the certified clean planting material prior to acclimatization in the greenhouse for field establishment and distribution to farmers (Otoo and Quain, 2001). The sweet potato, plantain and banana materials were also conserved *in vitro* using CSIR-CRI laboratory-optimized slow-growth protocols. This made the materials available for multiplication on demand.

Research at the CSIR-CRI tissue culture laboratory thus optimized the micropropagation methods for local plantain varieties (Quain *et al*., 2010). The developed biotechnology tools were used to produce clean planting material for field evaluation and dissemination of healthy planting material to farmers.

USE OF CLEAN PLANTING MATERIALS BY FARMERS – RESULTS, IMPACTS AND CHALLENGES

SWEET POTATO

Agriculture extension officers worked with researchers to disseminate clean planting materials to farmers by establishing a nursery under irrigation at regional agriculture stations and supplying the farmers routinely with healthy planting materials. To sustain the planting material multiplication and distribution system, the Roots and Tubers Improvement Programme adopted a nationwide three-stage strategy as practised in the Northern, Volta and Central Regions of the country:

- Primary planting material multiplication was done at research stations using tissue culture and sanitized planting materials under optimum agronomic conditions.
- Planting materials were transferred from the primary site to the secondary foundation planting materials multiplication site, which was managed by certified farmers under strict agronomic conditions.
- Certified planting materials were then distributed to farmers for direct use and further distributed to interested farmers.

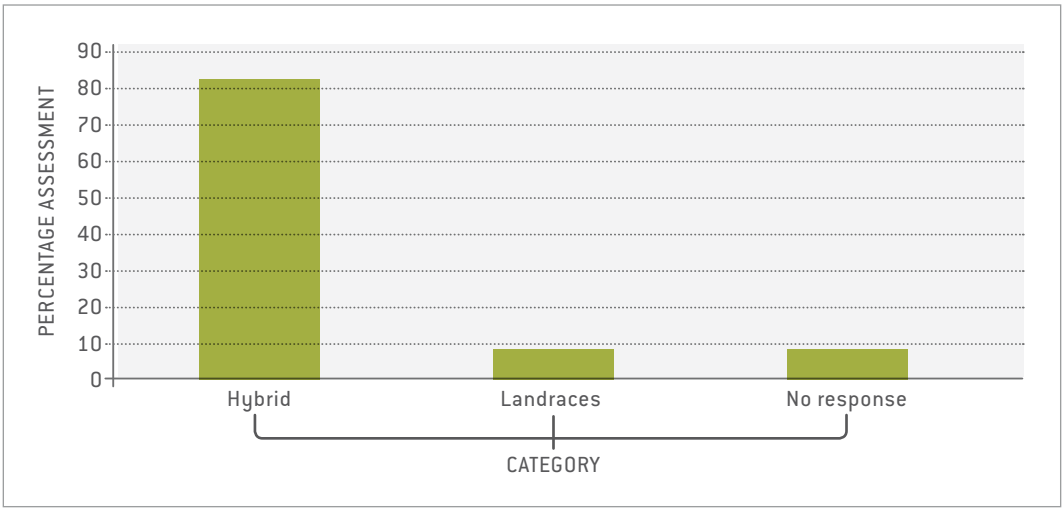
This system reportedly reduced disease pressure in fields so that there were healthier crop stands, which produced increased tuber yields of 12.3 percent and 30 percent for two released sweet potato varieties, Sauti and Faara, respectively (Otoo and Quain, 2001). These two varieties were established on 4.8 ha primary multiplication sites at the beginning of the

2002 planting season. Secondary multiplication sites were established on 35 ha, exceeding the target of 20 ha. By December 2002, a total of 1 209 500 vine cuttings had been distributed to 287 secondary farmers and, at the tertiary level, reached 14 500 resource-poor farmers. Yield studies on secondary multipliers in the Upper-East Region showed average yields of 11 t/ha for the Sauti and 13-15 t/ha for the Faara variety. The 14 500 farmers who adopted the new sweet potato varieties enjoyed output increases and ready markets for their produce, and so were likely to have increased their incomes (IFAD, 2004).

PLANTAIN AND BANANA

In a study to assess farmers' responses to tissue culture plantain planting materials, a total of 169 farmers from ten communities participated in a survey (Dzomeku *et al*., 2010). Of the total number of farmers interviewed, 111 were either direct beneficiaries or belonged to beneficiary households. Although most of the respondents were initially unfavourable to the tissue cultured seedlings, 84 percent finally declared the hybrids to be superior to the landraces in field establishment, plant growth and vigour (Figure 1). The farmers also reported that biotechnologically developed plantain hybrid plants were shorter than the landraces. Furthermore, the yield values of the hybrids were superior to the landraces. Farmers also added that the hybrids "stayed green" with about ten green leaves at harvest versus 0 to 4 for landraces. They reiterated that the "stay green" characteristic of the hybrids was an added advantage as they provided shade for their cocoa plants (Dzomeku *et al*., 2010).

Figure 1. Agronomic assessment of hybrids by farmers



source: Dzomeku *et al*., 2010

A farmer carrying hybrid bananas from her farm in the Assin District
©Beloved Mensah Dzomeku

In another study (Dzomeku *et al*., 2012), significant differences were found in the number of days to flowering, for fruit filling and to harvest between conventional sucker-derived and *in-vitro*-derived plants of banana (Table 1). They also found significant differences in banana plant height, with the tissue cultured plants producing taller plants which grew faster and whose pseudostem circumferences increased faster than those of the conventional sucker-derived plants during the vegetative growth period. Tissue culture plants with higher growth vigour are advantageous since stronger pseudostems mean plantations that can withstand stormy weather, during which plantain and banana plantations are usually devastated. The *in vitro*-propagated plants flowered about two weeks earlier than the sucker-derived plants. This was also reflected in the days to harvesting: the *in vitro*-propagated plants were harvested about 16 days earlier than the sucker-derived plants (Table 1). This is a very significant result, because it can guide farmers to target the lean-season harvest which also attracts higher prices. The faster growth of *in vitro*-propagated plants could be attributed to their intact active roots and shoot systems that can function almost immediately after planting, unlike in the conventional method where paring is done on the sucker before planting. There is therefore a lag phase in the sucker-derived plants, which requires two or more weeks for the sucker to start growth (Dzomeku *et al*., 2012).

Table 1. Yield characteristics of *in vitro*-propagated and sucker-derived banana plants under field conditions

PLANTING MATERIAL	DAYS TO FLOWERING	DAYS FOR FRUIT FILLING	DAYS TO HARVEST	BUNCH WEIGHT (T/HA)	No. HANDS	No. FINGERS
Sucker-derived	299.5 ±3	98.5 ±1	392.3 ±2	38.0 ±1	7.4 ±1	107.1 ±2
In vitro-propagated	286.2 ±1	90.3 ±4	376.5 ±5	39.1 ±2	7.5 ±1	104.2 ±3
P< 0.01	**	**	**	ns	ns	ns

ns= not significantly different ** significantly different at P<0.01. n=20

source: Dzomeku *et al*., 2012

As regards the food quality of the hybrids, studies have shown that they fit well into the Ghanaian diet (Dzomeku *et al*., 2007c; 2008a; 2008b). The performance of the hybrid bananas in juice production is reported to be better than the landraces (Asigri *et al*., 2008).

The introduction of *Musa* tissue culture planting materials met with some strong resistance from farmers. A study showed that over 70 percent of interviewed farmers who were introduced to tissue cultured seedlings of Musa were initially very apathetic towards them (Dzomeku *et al.*, 2010). They could not believe that plantain planting materials could be raised in polyethylene bags. They had also not seen tiny plantain planting materials before, and mistook the materials to be seedlings of garden flowers. However, after intensive education and strong assurances from the implementation team, the farmers reluctantly agreed to plant the varieties. Three months after planting, their attitude changed. They observed that the varieties were more robust than local varieties, and asked that they might be allowed to expand their cultivation in order to make enough profit from their farms before new farmers were enrolled in the project (Dzomeku *et al.*, 2010). The farmers were therefore reluctant to supply suckers for further deployment. In other cases, especially regarding farmers who used the dry plantain and banana leaves for "Fanti kenkey" production, the farmers complained that the "stay green" characteristic of the hybrids was affecting their business. They indicated that they were not getting enough dried leaves for their "kenkey" business (Dzomeku *et al.*, 2010).

A classroom teacher who was introduced to the production of banana juice from these FHIA hybrids as a pilot trial has now resigned from teaching and begun making juice as a full-time job. She supplies her products to supermarkets all over the country. The number of farmers growing FHIA-01 has increased tremendously over the years. Market women travel from Accra to Kumasi (over 270 km) to purchase fruits for sale in Accra. The yield of the hybrids far outweigh (by 30 percent) that of the local cultivars.

The protocol for *in vitro* production of plantain planting materials has since been used to produce clean planting materials of local accessions (Apantu, Apem, Oniaba and Osoboaso). Individuals, NGOs and religious organizations have purchased more than 2 000 seedlings for field establishment. Clean planting materials produced by tissue culture have now spread and are being grown in different regions of Ghana. The impacts of using the clean planting materials are illustrated in Table 2.

CSIR-CRI is a national research organization with a mandate to carry out research on crops for resource-poor farmers. The organization reaches farmers through agriculture extension officers. The main challenge has been to get farmers to acknowledge that their planting material is a product of CSIR-CRI research output and not the Ministry of Agriculture.

Table 2. Impact of adopting tissue culture derived planting materials

ATTRIBUTE	TISSUE CULTURE DERIVED CLEANED MATERIALS VERSUS THOSE FROM CONVENTIONAL METHODS (% INCREASE)	COMMENTS
Sweet potato tuber yield	30%	Added approximately 15% to farmers income
Sweet potato top yield	24.4%	Farmers have more planting materials for next season planting and were sure of source of planting materials
Plantain/Banana No. of hands	60%	Farmers have more healthy, uniform and clean planting materials
Plantain/Banana weight of hands	30%	Farmers have healthy and bigger fruits
Plantain plant height	10%	Robust and fast growing healthy plants
Banana plant height	10%	Robust and fast growing healthy plants
Plantain plant girth	10%	Robust girth
Banana plant girth	10%	Robust girth
Farmers adopting the use of clean planting materials	30%	Farmers demanding, but cannot afford, the real cost of tissue cultured planting materials

Funds are also not available to facilitate the follow-up of field evaluations once a sponsored programme is over. This is because planting materials are supplied free to the farmers. In recent instances where planting materials have been sold, the price was set very low and at no profit to the organization. It is therefore necessary to subject the *in vitro* propagation of clean planting materials to rigorous economic analysis, which will facilitate selling the findings to the private sector and generate more funds. The funds are needed to meet costs such as the hiring of labour at CSIR-CRI for *in vitro* rapid multiplication.

CONCLUSION

The application of biotechnology is needed to enhance smallholder agriculture productivity. Its use in the production of clean planting materials promotes crop establishment and vigour. The growth and yield of vegetatively propagated plants surpass those of the conventionally produced materials. It is evident that agricultural extension officers and smallholder farmers appreciate the reduction in plant disease pressures when clean healthy planting materials are established on their farms. This translates into enhanced incomes, job creation and improved livelihoods. Concerted efforts by the research community and government are seriously needed to promote the utilization of clean planting materials of clonally propagated crops in Ghana.

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Latin American cassava germplasm selected with markers for cassava mosaic disease (CMD) resistance showing good yield
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CHAPTER 2.4

MOLECULAR MARKERS AND TISSUE CULTURE: TECHNOLOGIES TRANSCENDING CONTINENTAL BARRIERS TO ADD VALUE AND IMPROVE PRODUCTIVITY OF CASSAVA IN AFRICA

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INTRODUCTION

Cassava (*Manihot esculenta* Crantz), though native to South America, is one of the most important food staples in sub-Saharan Africa (SSA), where it was introduced in the sixteenth century (Cock, 1985). Cultivated mostly for its starch storage roots in the tropical and subtropical regions of the world, Africa accounts for about 50 percent of the crop's annual global production, followed by Asia and South America, contributing about 30 and 20 percent respectively. Nigeria is the world's leading producer, with over 52 million metric tonnes (FAOSTAT, 2011).

Its ability to produce relatively more than other crops in marginal environments makes it a strategic crop for food security in Africa. With proper husbandry, cultivating cassava offers immense potential for enhanced income and improved livelihoods for the mostly small-scale farmers in SSA that grow the crop. Harnessed properly, cassava can therefore play a key role in rural development and growth in farming communities. In recent years it has gained importance as a cash crop in Africa.

However, for cassava's full potential to be realized, many constraints must be overcome. Yield is one of them, and there is much room for productivity improvement in SSA, so the yields can be at least comparable to those of South America and Asia. For instance, the average yield of fresh roots per hectare is 10.2 tons in Africa, 12.5 in South America, 17.3 in Asia and 12.4 worldwide (FAOSTAT, 2011). The development and dissemination of high-yielding well-adapted varieties that, in addition to meeting the requirements for nutrition and/or industrial applications, will also be suited to their agro-ecological and farming systems is critical to raising the crop's productivity in SSA.

EXPLORING AND ACCESSING VALUABLE CASSAVA GERMPLASM

A crop's germplasm is the repository for the genes used for developing superior varieties. Exploring cassava germplasm and deploying its widest possible heritable variation in developing improved, high-yielding and value-added varieties will be key to realizing this crop's potential. A major constraint to the breeding of improved cassava varieties is the limited access to useful germplasm. For instance, South America, the centre of genetic diversity for the crop with a wide array of genotypes including its wild relatives, is a veritable trove of heritable variations that can be used to improve the crop worldwide. Indeed, the international Center for Tropical Agriculture (CIAT) in Colombia has the largest cassava collection in the world, with over 6 000 accessions.

CIAT has utilized wild cassava relatives for genetic improvement of novel traits for which genetic variation is highly limited in cultivated gene pools.

It is evident, therefore, that access to South American germplasm is crucial to meeting the new emergent role of cassava as a cash and industrial crop in Africa. Important traits of interest for which useful genes are being sought for cassava in Africa include high dry-matter content, novel starch types, low cyanogenic potential, beta carotene content and delayed post-harvest physiological deterioration. The efficient use and rapid deployment of genetic resources from South America to Africa are central to current research efforts to meet the needs of farmers, processors and end users.

Severely curtailing the utility of the South American cassava gene pool is the fact that once cassava germplasm is transferred from this centre of diversity to Africa, it succumbs quite readily to the myriad virulent diseases and pests that are found there. A means for circumventing this drawback has therefore become imperative. The usefulness of biotechnological tools for mitigating such problems has been amply demonstrated in recent years. We describe how the combined applications of cell biology and molecular marker systems have enabled the introgression of novel desirable traits into cassava genotypes from the South American variants that ordinarily cannot be established in SSA, and how they are being incorporated into breeding programmes

CHALLENGES

The introduction of South American cassava germplasm into Africa is constrained by three main factors:

1. Their susceptibility to cassava mosaic disease (CMD);
2. Quarantine restrictions to the use of stem cuttings as propagules in the transfer of germplasm between the two continents; and
3. High rates of heterozygosity in cassava, an outcrossing species, meaning that progeny from botanical seeds are necessarily different from the parents.

CMD

This is the most important viral disease and a major constraint for cassava production in Africa and India (Patil and Fauquet, 2009). High CMD infection severely affects plant growth and development and leads to yield losses of between 20 and 95 percent (Fauquet and Fargette, 1990). These losses amount to billions of US$ annually.

CMD has not been reported in the Americas. Breeding for CMD resistance in the absence of the pathogen in this region was not possible even though the South American cassava germplasm tested in Africa was highly susceptible to CMD (Okogbenin *et al.*, 2007). The susceptibility of the introduced germplasm to CMD meant that its utilization in cassava genetic improvement could not be maximally exploited in Africa as the unadapted material could not be crossed with African germplasm (Blair *et al.,* 2007). Previous attempts to release Latin American genotypes as cultivars in Africa through collaborative partnership between the International Institute of Tropical Agriculture (IITA) and CIAT in the 1990s were equally unsuccessful due to their susceptibility to CMD.

QUARANTINE RESTRICTIONS

Cassava is affected by a plethora of pests and diseases. Given that cassava is vegetatively propagated with stem cuttings from year to year, diseases and pests rapidly build up in the planting materials, especially in susceptible cultivars. This accumulation of disease inoculum results in systemic infection and thus exacerbates the spread and high incidences of diseases. While overall concentrations tend to be low in resistant cultivars, they are nevertheless potent sources of inoculum from which the disease can spread (Fargette *et al.,* 1988).

There are several cassava diseases that are still restricted to different geographical regions of the world. For example, CMD is restricted to Africa and India while frog skin disease has been reported only in Latin America. The use of stem cuttings for germplasm exchange is therefore considered a high-risk method by plant quarantine authorities as it is prone to spread pests and diseases, hence the very strict restrictions on the use of these vegetative propagules in cross-border germplasm transfers. This severely limits the access of plant breeders to otherwise useful genetic resources that could serve as sources of novel traits.

HETEROZYGOSITY

The genetic improvement of cassava is complicated by the biology of the crop and its heterozygosity, which, in the absence of appropriate genetic stocks (inbred lines), has imposed limitations on the efforts to breed novel varieties. The cassava genome has a high genetic load, which means that there is a preponderance of unfavourable alleles in its genetic make-up. Due to this high level of heterozygosity, the progeny of normal bi-parental crosses manifest high rates of segregation and the seeds cannot be used efficiently for germplasm exchange because they do not breed true to type. The recovery of desirable trait combinations that are already fixed in clonally propagated materials in progeny derived from botanical seeds is so difficult, time-consuming and expensive that any such efforts are rendered impractical. Seeds are therefore not suitable for the transfer of cassava germplasm.

TISSUE CULTURE AND MOLECULAR MARKER TECHNOLOGIES

TISSUE CULTURE

Tissue (*in vitro*) culture is the growth of tissues and/or cells in a liquid, semi-solid or solid growth media under aseptic conditions. The development and validation of *in vitro* culture media protocols for cassava has greatly enhanced cassava germplasm transfer and contributed to circumventing the problems associated with the use of stem cuttings for cassava germplasm exchange between Latin America and Africa. Several hundred cassava genotypes, including interspecific hybrids (Table 1), have been transferred in this way with better phytosanitary status and reduced cost (Fregene *et al*., 2006). For example, over 30 000 *in vitro* culture plantlets representing over 700 genotypes (Table 1) were received by the National Root Crop Research Institute (NRCRI) in Nigeria from CIAT between 2004 and 2012. It has considerably minimized quarantine concerns over the introduction of diseases from one region to the other. This technique, which has proven to be a good strategy for maintaining good quality planting materials, has also been extended to rapid multiplication and the use of meristem tip culture for virus elimination from infected tissues.

Guided by the need to increase CMD resistance in Latin American germplasm, this technology

Table 1. CIAT shipment of CMD resistant genotypes, in combination with other traits, to Africa and Asia

COUNTRY	TRAIT	NUMBER OF GENOTYPES SHIPPED
Tanzania	r-CMD, r-CGM	530
Nigeria	r-CMD, r-CGM, RQ	765
Uganda	r-CMD, r-CGM	530
Ghana	r-CMD, r-CGM, RQ	765
Kenya	r-CMD, r-CGM, Dr	850
Mozambique	r-CMD, r-CGM, RQ	150
South Africa	r-CMD, r-CGM	80
India	r-CMD, r-CGM	530
Thailand	r-CMD, r-CGM	50

r-CMD, resistance to cassava mosaic disease; r-CGM, resistance to cassava green mite; RQ, root quality; Dr, dry matter

was exploited in transferring CMD-resistant African genotypes to CIAT, thereby permitting the use of African cassava genotypes as donor parents for CMD resistance in crosses with Latin American germplasm in Colombia.

In vitro culture is now the preferred means for germplasm transfer, and has greatly aided greater access by breeders to germplasm. By shipping several copies of a genotype via *in vitro* culture, germplasm loss is minimized and sufficient planting materials of genotypes can be generated quickly for breeding trials.

MOLECULAR MARKERS

Molecular markers are valuable tools for understanding genetic variation. They identify differences at the DNA level and have been applied to the analysis and discovery of genes for CMD resistance and other important traits in cassava.

Germplasm introduced from CIAT to Africa in the 1990s was completely devastated by CMD and none was released as a variety in the 30 years of unsuccessful attempts. When African CMD-resistant donor lines of *Manihot glaziovii* were used in crosses with Latin American clones in Colombia to improve CMD resistance, resistance was not fully transferred from the donor clones because of the polygenic and recessive nature of its inheritance.

The search for a new source of CMD resistance by IITA and CIAT resulted in the discovery of high resistance in a Nigerian landrace (TME3). Classical genetic studies indicated that the high resistance in TME3 was due to a dominant gene and the use of molecular marker technology led to genetic mapping of the gene, called *CMD2* (Akano *et al*., 2002). The dominant genetic nature of *CMD2* means that CMD resistance can now be transferred and tracked easily by molecular markers and that breeding in the absence of the pathogen can be implemented.

Markers associated with *CMD2* were efficiently used to introgress CMD resistance into Latin American germplasm in CIAT (Fregene *et al*., 2006). This enhanced the adaptation of Latin American germplasm in Africa. Once introduced to Africa, these exotics could be crossed with the locally adapted materials thereby paving the way to introducing into the African cassava gene pool other desirable novel traits inherent in the Latin American germplasm.

The CMD molecular markers used to trace inheritance of the genome segment contributing to CMD resistance are therefore now fast-tracking the use and release of Latin American genotypes as new varieties in Africa. The strategy entails using markers to preselect neotropical Latin American cassava genotypes for CMD resistance in the Americas. The selected genotypes are then evaluated for 1 or 2 years in Latin America before being shipped to Africa where they are evaluated for 3 to 4 years on station and in multisite trials with the best genotypes released as varieties. Based on the validated protocols, it takes about 5 or 6 years for elite clones developed from exotic germplasm to be released to farmers (Okogbenin *et al*., 2007).

A Latin American cassava cultivar, CR41-10 (UMUCASS 33), selected using CMD resistance markers was released in 2010 in Nigeria after 6 years of work and represents the first Latin American cultivar to be released in Africa. The cultivar was selected by farmers for its culinary quality and good architecture that makes it well suited to the cropping systems used by smallholder farmers. Similarly, another variety, CR36-5, was released in 2012 for high starch content (27.1 percent, see Table 2) as required by starch and high-quality cassava flour industries in Nigeria. This development is a landmark breakthrough in attempts to provide farmers with good varieties from the crop's centre of origin in addition to increasing the genetic diversity in the farmer's field. Both released varieties are resistant or tolerant to other important pests and diseases (cassava bacterial blight, cassava anthracnose, cassava green mite and cassava mealybug). They also have very good fresh root yield of 46.6 t/ha (for CR41-10) and 42 t/ha (for CR36-5).

Table 2. Starch content of improved cassava varieties grown in Nigeria

VARIETY	STARCH (%)
NR 03/0155	22.80
NR 03/0211	22.20
TMS98/0581	24.51
TMS98/0510	22.71
TMS01/0040	19.26
TMS30572 (national check)	21.50
CR36-5	27.10

Typically, in hotspot zones for the disease, susceptible genotypes can be easily identified within 2 to 6 months after planting. However, the challenge for the breeder is often not in identifying the susceptible genotypes but in selecting genotypes with durable and stable resistance, which basically requires field screening for at least three years. Because cassava is vegetatively propagated, genotypes with mild or moderate resistance might deceptively appear resistant in the first year, but as the inoculum builds up in the vegetative planting materials, there is the tendency for an increase in the disease severity for such genotypes. Eliminating such genotypes in the first year to reduce cost is best achieved with the aid of markers. As new sources of CMD resistance are identified, the need to select for high resistance and gene pyramiding would make marker-aided breeding for CMD resistance the best option and almost inevitable.

NEW FRONTIERS AND OPPORTUNITIES FOR AFRICAN FARMERS

A key target for breeding is to ensure that farmers have access to the right varieties they need to meet food and commercial purposes. Access to Latin American germplasm provides an array of opportunities for farmers to meet these demands, especially with respect to quality (value added) and productivity traits.

Under the Consultative Group on International Agricultural Research (CGIAR) Generation Challenge Programme initiative, Latin American germplasm introgressed with CMD resistance and developed for characters such as high yield, vigour, high dry-matter content, high starch content and drought tolerance are now accessible to breeding programmes in Nigeria. Molecular markers have been used to introgress CMD resistance into backcross derivatives of wild relatives developed for novel traits and then introduced into Nigeria to improve value addition in cassava. These include high nitrogen content in roots (potentially for high protein content) and delayed post-harvest physiological deterioration. The introduced germplasm have been incorporated into the NRCRI-curated cassava gene pools, and is now available for continuous use in breeding programmes.

A Latin American (CIAT) variety called CR36-5 showing good resistance to CMD, released in Nigeria in 2012
©Emmanuel Okogbenin

PREDICTED IMPACTS

Ex-ante impact assessment studies indicate that cultivars developed with marker-assisted breeding that incorporate pest and disease resistance as well as quality traits could be worth US$2.89 billion in Nigeria over 20 years. If developed for pest and disease resistance alone, they would be worth US$1.49 billion. When developed solely by conventional breeding they would be worth about US$676 million in Nigeria. The difference is mostly due to the faster timing of release for the cultivars developed with markers and the higher probability of success (Rudi *et al.,* 2010).

MAS-developed released varieties with good adaptation have the potential to enhance yield increases in Nigeria from the average 14 t/ha to 25 t/ha being targeted by the Cassava Transformation Agenda of the Nigerian Government with an estimated additional revenue of 1.48 billion US$ for the cassava sector. Two starch mills in Nigeria have a combined capacity of 20 000 metric tonnes starch but operate under full capacity. The use of high-starch varieties can reduce current gaps in starch demand. Anticipated adoption for the high-starch variety released is put at 50-60 percent. The government is planning 18 new high-quality cassava flour (HQCF) factories with a total capacity of 1.3 million metric tonnes (Mt). Boosting HQCF needs with high starch varieties will save an estimated US$2 billion annually by creating a market of 1.6 Mt (6.4 million Mt of fresh root cassava) and thousands of jobs (Fregene, personal communication).

CONCLUSION

The introgression of CMD resistance using molecular markers into Latin American cassava genotypes and their subsequent transfer as *in vitro* cultures to Nigeria and other African countries has improved the access of breeders and farmers to useful germplasm required to broaden the genetic base of the crop on the continent. The combined use of CMD molecular markers and *in vitro* culture has also markedly reduced the population sizes of imported Latin American germplasm, as only the potentially CMD-resistant germplasm identified with molecular markers is shipped. This translates into significant reductions in cost to national agricultural research systems (NARS) breeding programmes. Through biotechnology interventions, the capacity of NARS to adopt modern breeding techniques for value addition, increased productivity and commercialization of the crop has been strongly enhanced.

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Plantain cultivar 'INIVIT PV 06 - 30' propagated by somatic embryogenesis and cultivated by farmer cooperatives
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CHAPTER 2.5

SOMATIC EMBRYOGENESIS FOR THE PRODUCTION OF PLANTAIN PLANTING MATERIALS IN CUBA

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SUMMARY

Bananas and plantains are major food crops for millions of people in tropical developing countries where the fruit is an important component in the diet. In Cuba, this crop is given a high priority in the national food programme because of its capacity for producing fruit all year round, the high demand and diversity of use. However, the available planting materials, i.e. suckers, produced by commercial laboratories (known in Cuba as biofactories) using the plant's axillary buds are insufficient to meet the demands of farmers. To address the perennial shortfalls in the supply of planting materials, an alternative robust higher throughput production system had to be devised. Relying on the totipotency of plant cells, i.e. the capacity of individual cells to regenerate a whole plant, somatic embryogenesis, whereby a whole plant or embryo is derived from a single somatic cell or group of cells, has been successfully used to obtain higher quantities of planting materials per unit time and cost. We describe the validated plant regeneration protocol via somatic embryogenesis for the AAB *Musa* group. This protocol has since been scaled up for commercial production of planting materials. Six biofactories in Cuba currently use somatic embryogenesis to produce planting materials which have been evaluated by farmers under field conditions. The genetic stability of regenerated plants and high yields obtained under field conditions demonstrate the feasibility of scaling up this biotechnological protocol and adapting it to commercial production of planting materials to mitigate a critical bottleneck in the value chain of this important crop.

INTRODUCTION

In Cuba, plantain is a high-priority crop in the national food system especially on account of its ability to produce all year round coupled with its high per capita consumption rate and the diversity of its use. However, due to the prevailing low yields attributed principally to attacks of "Black Sigatoka" disease, caused by *Mycosphaerella fijiensis,* and poor quality planting materials – usually a means for transmitting this disease from one generation to the next – the plantains (called "plátanos machos" in Cuba, of the AAB group) were gradually replaced by other more resistant variants, such as cooking bananas of the ABB *Musa* group and tetraploid hybrids developed by the Fundación Hondureña de Investigación Agrícola (FHIA), even though they are of lower acceptance.

Researchers and growers were therefore motivated to find alternative means of producing planting materials in order to reverse the decline in the production of plantain in the country and hence also stabilize the market. This became a priority research theme at the Research

Institute of Tropical Root and Tuber Crops (INIVIT) in the Province of Villa Clara in Cuba. Addressed principally through a PhD research project, a plant regeneration methodology using somatic embryogenesis has been developed, validated and is now routinely applied in the *in vitro* propagation of AAB *Musa,* i.e. plantains. Of note, the scaling-up of the protocols for commercial production of the planting materials was made possible by the enthusiastic adoption of the methodologies by commercial entities. To enhance adoption by farmers, field trials of the ensuing planting materials were carried out on-farm. We review below the steps in the development of this methodology for the production of planting materials and the dissemination of the research outputs.

RESEARCH AND DEVELOPMENT

The development of the protocol for high throughput regeneration of plantain planting materials via somatic embryogenesis has been described in detail by López (2006). The methodology involves a series of sequential stages ranging from obtaining explants and callus induction to formation, maturation and germination of embryos and, finally, conversion into plants and field production. These specific stages include:

a. Selection of suitable plants in the field as sources of explants;
b. Shoot apices of axillary buds – i.e. explants – were excised;
c. The explants were induced to form callus on appropriate aseptic growth media;
d. Embryogenic structures were isolated;
e. Embryogenic calli were multiplied in cell suspension cultures;
f. Embryogenic cell suspensions were induced to obtain somatic embryos;
g. Somatic embryos were left to mature;
h. Mature embryos were induced to germinate
i. Plantlets were established in the greenhouses for acclimatization;
j. Hardy plants were transferred to the field for observation.

Based on the above protocol, the methodology was applied to Cuban plantain genotypes, especially 'CEMSA ¾' and 'INIVIT PV 06 – 30' (a recently developed variety). Once embryos were obtained at the INIVIT laboratory, they were transferred for scaling-up to six biofactories (commercial laboratories) – three in the Villa Clara Province and one each in the Provinces of Cienfuegos, Sancti Spíritus and Ciego de Ávila.

The germination of embryos and their subsequent conversion to plantlets as described above were evaluated for agronomic traits in comparison to plants that had been established through the conventional means of corm buds that had been sourced from plantain plantations. These trials were aimed at evaluating the efficacy and sustainability of the use of *in vitro* – as against conventional - techniques in producing plantain planting materials. These trials were carried out on farms managed by individual small-scale farmers or on larger farms managed by farmer cooperatives, e.g. the Service-Credit Cooperatives (CCS by its Spanish acronym). Of note was the innovative collaboration between researchers and end users. Significantly, the obtaining of the somatic embryos was funded by the public institution, INIVIT, and these embryos were provided at no cost to the biofactories which, in turn, financed multiplication of the embryos and the eventual induction of plantlets. The hardened plants were subsequently provided cost-free to the farmers for field evaluation.

RESULTS

SCALING-UP OF PROPAGATION BY SOMATIC EMBRYOGENESIS IN BIOFACTORIES

It took just over one year, under the laboratory conditions of INIVIT, to generate mature embryos from explants (Table 1). Interestingly, almost half of all the induced somatic embryos formed mature embryos, at which point they were distributed to the biofactories. A significant majority (85 percent) of the mature embryos received at the biofactories germinated and almost all the germinated plantlets (97 percent) were successfully acclimatized. The germination and acclimatization phases in the biofactories required an additional maximum of four months. Regarding efficiency, somatic embryogenesis makes it possible to produce large volumes of plant material in a short amount of time. Once the cell suspension has been established, it can be multiplied every 15 days and just 2-3 drops of the cell suspension are needed to yield an average of 2 500 embryos from which almost 1 000 plants are recovered as hardened plantlets ready for field establishment (Table 1). If needed, the method allows the biofactory to plan for the production of very large volumes of plant materials with the only real limitation being the laboratory glassware. By comparison, when propagation is by organogenesis from meristematic apices, results showed that a maximum of 5 000 plants can be produced in a year from a meristematic apex.

In the biofactories, production of planting materials through somatic embryogenesis yielded higher multiplication coefficients than *in vitro* regeneration using meristematic apices.

Table 1. The duration and yields of the different stages of somatic embryogenesis in plantain

DEVELOPMENT STAGE	RESULTS	DURATION (MONTHS)
BIOTECHNOLOGY LABORATORY		
Obtaining explants	Multiplication of shoot apices of axillary buds	3
Induction of somatic embryogenesis	Calli with embryogenic structures (20-30% according to genotype)	3
Establishment of embryogenic cell suspensions	Established suspension with 27.8 x 10^4 embryogenic aggregates with rapid sedimentation	1
Multiplication of embryogenic cell suspensions	Cell biomass increase of 1.5 – 2 ml per 100 ml Erlenmeyer flask every 15 days	2-3
Somatic embryo formation	2 500 embryos in two or three drops of cellular suspension	1½
Maturing of somatic embryos	1 200 mature embryos (48%)	1
BIOFACTORIES		
Embryo germination	1 020 germinated embryos (85%)	1-2
Plantlet acclimatization	987 plantlets ready to be planted in field conditions (97% surviving)	2

Comparisons effectively demonstrate the relative higher multiplication ratio of somatic embryogenesis over other methods traditionally used in commercial laboratories for producing planting materials of vegetatively propagated plants. The INIVIT model also demonstrates that only the more technically challenging upstream activities need be performed in the laboratory setting. Multiplication of the planting materials, which requires more space, can be farmed out to commercial entities, in this case biofactories, without any loss in efficiency.

FARMER-FIELD LEVEL VALIDATION OF THE SOMATIC EMBRYOGENESIS METHODOLOGY FOR GENERATING PLANTING MATERIALS OF PLANTAIN

A total of 43 534 plants, obtained from the somatic embryos supplied to six biofactories, were evaluated in 10 different farmer-managed fields in four provinces of Cuba (Table 2).

Plants from somatic embryos showed similar growth habits to plants obtained from meristematic apices (organogenesis) and corm buds, irrespective of the cultivar evaluated and the location of the evaluation.

Table 2. Biofactories and farmer cooperatives that collaborated in the production via somatic embryogenesis and field trialling of plantain planting materials

PROVINCE	BIOFACTORY	FARMERS COOPERATIVE	CULTIVAR	PLANTS OBTAINED
Villa Clara	Santa Clara	Jorge Mazo CCS "Pedro J. Marcelo"	'INIVIT PV 06 – 30'	500
Villa Clara	Santa Clara	State sector	'INIVIT PV 06 – 30'	2 500
Villa Clara	INIVIT	Alberto Vázquez; CCS "David Díaz"	'INIVIT PV 06 – 30'	5 000
Villa Clara	Centro de Desarrollo	Carlos León; CCS "Cuba Viet – Nam"	'CEMSA ¾'	3 000
Cienfuegos	Cienfuegos	Michel Rumbaut; CCS "Ernesto Che Guevara"	'INIVIT PV 06 – 30'	8 000
Cienfuegos	Cienfuegos	José Quintana; CCS "Manuel Ascunse"	'INIVIT PV 06 – 30'	4 000
Sancti Spíritus	Sancti Spíritus	Ulises Orellana; CCS "Enrique Martínez"	'INIVIT PV 06 – 30'	15 000
Ciego de Ávila	Ciego de Ávila	State sector	'INIVIT PV 06 – 30'	2 227
Ciego de Ávila	Ciego de Ávila	CCS "Máximo Gómez"	'INIVIT PV 06 – 30'	1 640
Ciego de Ávila	Ciego de Ávila	State sector	'INIVIT PV 06 – 30'	1 667
TOTAL				**43 534**

The frequency of somaclonal variation observed among the progeny from somatic embryos evaluated in Villa Clara and Cienfuegos provinces was lower than 2 percent. This negligible frequency implies that plantain can be effectively propagated through somatic embryogenesis without any significant loss of fidelity from parent to offspring (Table 3).

In assessing bunch characteristics, as major yield components, plants regenerated from somatic embryos had similar values to those regenerated through organogenesis for all variables evaluated while showing a higher performance than the plants established from corm buds (Table 4). Average bunch weights of 10.45 to 10.23 kg per plant were obtained for plants regenerated via somatic embryogenesis and organogenesis respectively. Both values were significantly higher than the average bunch weight for plants established from corm buds (8.1 kg).

In the evaluation of economic indicators based on the returns from one hectare planted with the 'CEMSA ¾' cultivar in the CCS "Cuba - Viet Nam" (Table 5), the highest net profit ($22 294 Cuban pesos) was determined for plants produced through somatic embryogenesis. This exceeded by $62 Cuban pesos the net profit realized from plants obtained through organogenesis (apical meristems) and by $7 585 Cuban pesos the plants whose planting materials were sourced from corm buds. These demonstrate the superiority, in terms of weight gain, profitability and crop yields, of the planting materials produced through somatic embryogenesis over those sourced from alternative means (Table 6).

Table 3. **Percentage of phenotypic variants obtained through propagation by somatic embryogenesis and organogenesis during the first growing cycle in field conditions**

PHENOTYPIC VARIANTS /CULTIVAR	VILLA CLARA PROVINCE						CIENFUEGOS PROVINCE		
	'CEMSA ¾'			'INIVIT PV 06 – 30'			'INIVIT PV 06 – 30'		
	SE	Org	Corm	SE	Org	Corm	SE	Org	Corm
Variegated leaves	0.4	0.6	0	0.3	0.4	0	0.7	0.9	0
Change of pseudostem colour	0.4	0.2	0.6	0.2	0.3	0	0.2	0.1	0
Thin pseudostem	0.3	0	0	0.1	0	0	0.2	0	0
Regression to French type	0	0	0	0.1	0.2	0.1	0.3	0.2	0.9
TOTAL CHANGES	**1.1**	**0.8**	**0.6**	**0.7**	**0.9**	**0.1**	**1.4**	**1.2**	**0.9**

Legend: SE, somatic embryogenesis; Org, organogenesis.

Table 4. **Bunch characteristics of plants obtained by somatic embryogenesis, organogenesis and corm buds during the first growing cycle at CCS "Cuba – Viet Nam"**

TREATMENTS	BUNCH WEIGHT (KG)	HAND NUMBER	FINGER NUMBER
Somatic embryogenesis	10.45a	7.04a	37.52a
Organogenesis	10.23a	7.04a	37.32a
Corm buds	8.10b	4.80b	31.56b

Means with different letters in the same column are statistically different at $p < 0.05$ according to Dunnett's C test.

Table 5. **Estimates of economic indicators for plantain fields established with three different planting materials based on one hectare of cultivar 'CEMSA ¾'**

TREATMENTS	TOTAL COST ($)	NET INCOME ($)	NET PROFIT ($)	PROFITABILITY (%)	COST PER WEIGHT ($)	COST PER TONS
Corm buds	3 451.00	18 160.15	14 709.15	710.38	0.32	852.00
Organogenesis	3 092.30	25 324.18	22 231.88	1 198.23	0.20	604.55
Somatic embryogenesis	3 092.30	25 386.51	22 294.21	1 201.60	0.20	591.83

Table 6. **Economic efficiency achieved from different "seed" sources used for planting the 'CEMSA ¾' cultivar in a hectare**

TREATMENTS	YIELD (TON)	INCREASED YIELD (TON)	PROFIT INCREASE ($)
Corm buds	6.750	_	_
Organogenesis	8.525	1.775	7 522.73
Somatic embryogenesis	8.708	1.958	7 585.07

As seen in Table 7, the 'INIVIT PV 06 – 30' cultivar also showed similar performance in bunch weight evaluated with no significant differences between *in vitro* propagated plants, but significant differences were noticed in relation to field propagated plants.

Table 7. Bunch characteristics of plants from the cultivar 'INIVIT PV 06 – 30' obtained by somatic embryogenesis, organogenesis and corm buds during the first growing cycle at CCS "Ernesto Che Guevara"

TREATMENTS	BUNCH WEIGHT	HAND NUMBER	FINGER NUMBER
Somatic embryogenesis	18.84a	6.54a	41.17a
Organogenesis	19.51a	6.37a	42.21a
Corm buds	12.07b	6.29a	33.42b

Means with different letters in the same column are statistically different at $p < 0.05$ according to Dunnett's C test.

Evaluations carried out in other locations also showed the superiority of the proposed methodology (data not shown). The scaling-up of the methodology developed for banana propagation by somatic embryogenesis ensures the genetic stability and harvested bunch quality in regenerated plants and corroborates the possibility of multiplying target cultivars by somatic embryogenesis. All of which also was obtained in the cultivar 'Navolean' (López *et al*, 2005).

All the above has been endorsed by economic indicators. Moreover, it is important to consider that the application of the methodology is not intended to substitute but rather to complement the propagation method via organogenesis that is performed in biofactories, because "seed" volumes are insufficient in this crop in Cuba. Another important aspect to consider is that the majority of plantains from group AAB are planted by the extra dense planting system which requires more plants for field planting during a crop cycle.

In conclusion,

1. Plant regeneration by somatic embryogenesis provides another alternative for *in vitro* propagation from embryogenic cells. The feasibility of propagating the 'CEMSA ¾' and 'INIVIT PV 06 – 30' plantain cultivars by this method was determined.
2. The scaling-up of propagation by somatic embryogenesis was possible from a research laboratory to another production laboratory (biofactory) through maturation and somatic embryos conversion, in addition to multiplying them via organogenesis, demonstrating the superiority of the developed methodology by the plant numbers obtained in less time.
3. Field observations and evaluations by farmers showed the possibility of using this propagation method, due to the high yields obtained and the genetic stability of propagated plants.

Plantain cultivar 'INIVIT PV 06 - 30' propagated by somatic embryogenesis and cultivated by farmer cooperatives
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A woman farmer doing tissue culture at the rural laboratory, using a low-cost flow cabinet to prevent contamination

CHAPTER 2.6

USE OF TISSUE CULTURE IN CASSAVA FOR RURAL HOUSEHOLDS IN COLOMBIA

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Cassava (*Manihot esculenta* Crantz) originated in tropical America and is widely grown as a subsistence crop by small-scale farmers. Although cassava usually grows under agricultural limiting factors, such as low soil fertility, soil acidity, water deficit and a minimum of farm inputs, it has become a dependable food crop for resource-poor farmers. Families or community-based agricultural production systems with limited financial resources are common in countries like Colombia in South America. Under special circumstances, farmers are able to use minimal doses of fertilizer to obtain increases in yield and income.

Cassava is a clonal crop, reproduced by stem cuttings. This propagation method favours the dissemination of systemic pests and pathogens. For example, the occurrence of the economically significant frog skin disease (FSD) constitutes a major constraint to cassava production in Colombia. Recently, this problem has been reported in Panama, Costa Rica, Ecuador and Paraguay.

Tissue culture can contribute to the control of FSD by generating quality planting material of farmers' preferred varieties. Lack of good quality planting material of farmers' preferred cassava varieties constitutes a major constraint to the expansion of production in most of Latin America. To approach this problem, our work has focused on developing an "informal farmer's seeds production system" to be implemented in rural areas with low inputs through the implementation of a low-cost tissue culture laboratory. Good quality planting material refers to a system that combines key factors such as freedom from pests and diseases (i.e. an acceptable phytosanitary level); lack of varietal mixtures (i.e. a good genetics quality); and cost-effectiveness (a good relationship between propagation rate and cost, i.e. efficiency vs investment). Establishing local *in vitro* seed banks, handled by farmers, provides the required ownership of the system to local farmer communities.

The initial intervention area in our work was carried out in Colombia's Cauca Department where cassava is primarily utilized for starch extraction under local artisanal production systems called "rayanderias", which supply nearly 80 percent of the national demand for sour starch, especially for the bakery industry. In the last five years, a project on biodegradable plastics was carried out in the region, providing farmers with alternative markets for the harvested cassava roots. However, because of periodic variations in the cassava market price, it was not feasible to establish a reliable, programmable production scheme for the crop (G. Jaramillo, personal communication, 2004). Often, local actors introduce roots from other Colombian cassava-growing regions, or from Ecuador, at a lower cost. In addition, the Colombian Cauca region has been the scene of protracted social unrest which exacerbated inequality and contributed to the low impact of government social programmes and the aid programmes of local and international agencies.

Knowledge-sharing has been the basis of our project, where each actor contributes to an specific activity, i.e., the Women Farmers Group from Santa Ana community (ASOPROSA) is a target group of cassava experts; an NGO (Fundación para la Investigación y Desarrollo Agrícola, FIDAR) supports social work and personnel relationships; a Consultative Group on International Agricultural Research (CGIAR) member (the International Center for Tropical Agriculture, CIAT) provides experts in tissue culture and participatory research methodologies and the management of an *in vitro* gene bank, and financial support agencies (the Cassava Biotechnology Network [CBN], and Participatory Research and Gender Analysis [PRGA] Program from CIAT). The local cassava variety named Algodona (CIAT's code COL 1522) was used to adjust the methodology. Algodona is preferred by farmers due to its starch quality, in spite of being susceptible to diseases (Escobar *et al*., 2006). Additionally, five other clones were used to validate the tissue culture process.

In the 1970s, CIAT developed a tissue culture method for growing apical shoot tips on sterile culture media (Roca, 1984). Based on this method, we adapted and developed a simpler protocol using local components as culture medium reagents like locally available fertilizers, fruits juices, table sugar, cassava starch among others; and low-cost tools such as insulin syringes to be used as micropipettes, household scales, spoons, stove and pressure cookers. For tissue culture practice, it is necessary to get access to a flow cabinet that for the implementation of a rural laboratory could be expensive. For this reason, it was necessary to build a cheaper system that allows the maintenance of sterility conditions (Escobar *et al*., 2004).

A rural laboratory was designed and built with emphasis on its functionality, including different spaces for use as a propagation or growth room, kitchen for growth medium preparation and a yard for plants recovery. To develop this programme, it was necessary to establish simple communication through a language of terms connecting technical and local jargon. A farmer from the region was selected and trained to help in bridging farmers with technicians. Communication improved after days of practice. Farmer-farmer training, i.e. doing by themselves and interacting with conventional technicians, allowed the local women's group to develop tissue culture skills and procedures.

Among the technical problems encountered when going through the process of placing the system in farmers' fields, some were obvious, such as the lack of infrastructure, poor access to roads, non-appropriate laboratories and presence of outlaw activists. Some were less obvious, such as non-acceptance or lack of knowledge concerning sterility conditions, because farmers did not realize that certain microorganisms could cause harm or even kill plants. This issue was linked to their learning and acceptance of the need to use clean practices on a routine basis,

as well as the need to remove domestic animals from the laboratory area. Further, insufficient schooling and reading abilities were detected in some of the participants. At a somewhat different level, the time that women participants had to dedicate to the process had to be adapted to the time spent on other household responsibilities such as taking care of children or going to the local market. In view of the women's priority responsibilities, their schedule in the laboratory had to be adjusted to start at 14:00. In other instances, we had to respect their timing around noon for watching their preferred TV show.

Once the laboratory was completed and well established, a training programme was conducted to develop particular skills. For example, to cut down on the contamination rates of tissue cultures or to implement propagation schemes in the rural laboratory (i.e. including activities such as *in vitro* propagation, hardening and transfer to the field). Gaining knowledge about farmers with special abilities was crucial for the implementation of the rural laboratory. For example, women with vaccination practice could take responsibility for medium preparation or recognition of volume by microlitres using syringes for measuring culture media. Interestingly, school-age children helped their mothers with the reading and overall translation of written laboratory procedures.

A major conclusion of team work (farmers and technicians) is that all actors need to understand that information-sharing moves in both directions, which enhances knowledge and facilitates the process. When it is necessary to improve a given skill, the person is placed on front of a partner to facilitate communication and learning. An effective programme must ensure that farmers have a strong voice throughout the range of activities. In particular, farmers and their communities should help to define and re-define goals, weigh the different options to be tested, evaluate options actively and have their feedback taken seriously. Farmers also have to take steps to learn the concepts and incorporate appropriate insights of other partners (Escobar *et al.*, 2006).

Based on this experience, CIAT with FIDAR and other local partners, the Corporación para el Desarrollo Participativo y Sostenible de los Pequeños Agricultores Colombianos (Corporación PBA) and the Colombian Corporation for Agricultural Research (Corpoica), have been spreading the technology to some farmers associations (Asociación Municipal de Usuarios Campesinos [AMUC] at Santander de Quilichao, Cauca Department; Asociación Municipal para el Desarrollo Sostenible de los Pequeños Agricultores [ASOMUDEPAS] at San Jacinto, Bolivar Department; and the Empresa Comunitaria of San Rafael at Ovejas, Sucre Department), basically using cassava and yam as key crops (Escobar *et al.*, 2008). In five Colombian departments, we developed different initiatives, at the farmer- or rural school-level. They included three projects for farmer's laboratories, five farmers association that use/receive tissue culture material to

make different test (i.e. to renew/refresh planting material or to make a test for FSD behaviour) and three rural school laboratory projects. A total of 119 farmers were involved in those projects.

In a collaborative project among CIAT and the Corporación para Estudios Interdisciplinarios y Accesoria Técnica (CETEC), a Colombian organization that provides technical assistance to starch-producing farmers, comparisons were made of two different origins of Algodonas plant material (i.e. *in vitro* and conventional cuttings from farmers' fields) at Caldono, Cauca Department. Farmers observed that roots harvested from *in vitro* material are bigger, wider and heavier than conventional sources. As Marino Erazo, a farmer from CETEC, observed: "not all cuttings from our field, produce roots (i.e. without yield)", in comparison with the *in vitro* plot (i.e. "entire harvest of *in vitro* material produces roots"). The average yield of 300 *in vitro* Algodonas plants was 7.5 kg/plant, similar to data reported by ASOMUDEPAS farmers in the north coast of Colombia with other clones adapted to this region. This last group has developed a venture with seeds, and last year produced material for 200 farmers with a final price of 26 Colombian pesos per cutting (1 700 Colombian pesos = 1 US$) or 800 Colombian pesos for hardened material (R. Quiros. personal communication, 2013). The CETEC farmers' groups were able to recover 4 500 new cuttings from 300 initial planting material provided by the project, which formed the basis for a new plantation on two farms for the establishment in 2013 a community seed bank for associated farmers.

With the financial support of the Colombian Agricultural and Rural Ministry and the Japanese Embassy in Colombia, rural schools in Caracol, Sucre Department, were targeted as a means of integrating and diffusing biotechnology in the biology curriculum (Escobar *et al.*, 2010a, 2010b). As of 2013, two new initiatives are ongoing. The first, with the financial support of the Japanese Embassy, is a project for rural school strengthening that will start to build the laboratory facilities at Piendamo's school in Cauca Department that integrates other small schools. The second, with financial support from the Fondo Regional de Tecnología Agropecuaria (FONTAGRO), is a project that focuses on the release of tissue culture planting material free of FSD with a differential response and on-site implementation of macro-propagation scaling-up in the Guechené and Morales farmers' group in the Cauca Department and Granada, Meta Department.

The implementation of social programmes involving environmental training and participatory research develops conditions for the integration of biotechnologies in the context of smallholder agriculture, with crops and traits that are relevant to local farming constraints. An immediate benefit has been the restoration of intra-crop diversity that had been lost or decreased as a result of extreme weather events, pest and disease attack, or social unrest (Escobar and Roca, 2013).

Rural school tissue culture facilities: Practising the transfer of plantlets to substrate, with the participation of teachers and students of different ages
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Our results show concrete benefits that can improve the living conditions of resource-poor farmers through the adaptation of biotechnologies with minimal inputs for the production of clonal "seed". At the end of the process, an outstanding learning lesson was that farmers could adapt the meaning of tissue culture to their jargon, using language such as "propagation in little jars (*in vitro*) consists in planting heads or small trunks of the plant in particular food (growth media) so that they can grow and form new plants again", (comment by Hilda Castillo, a farmer from ASOPROSA). But above all, the final products of this technique are the cassava roots, no different from the roots we know ("what good cassava from those little jars, very soft"). Building local capacities and management skills have contributed to enhancing local food security and opened opportunities for the development of rural enterprises and for the restoration of local agrobiodiversity.

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Swarna-Sub1 (left) survived 10 days of floods during the vegetative stage, while Pooja (right) did not. Photo taken in Puri, Odisha in 2012, one month after the flood receded
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CHAPTER 2.7

TRANSFORMING RICE PRODUCTION IN FLOOD-AFFECTED AREAS: DEVELOPMENT OF THE SWARNA-SUB1 VARIETY USING MARKER-ASSISTED BACKCROSSING AND ITS DEPLOYMENT IN INDIA

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Rice in India is grown on 45 M ha; about 16 M ha is rainfed lowland of which 5.36 M ha is prone to submergence. These areas are highly populated with impoverished communities and with limited livelihood options. Modern rice varieties cannot withstand submergence beyond 4-5 days, causing low yields, averaging only about 0.5 to 0.8 t/ha in flood-prone areas, and sometimes crops are completely lost when floods are severe. The frequency and severity of poverty and food insecurity in these areas are high.

DISCOVERY OF FLOOD TOLERANCE IN RICE AND ATTEMPTS TO DEVELOP TOLERANT VARIETIES

Farmers in eastern India had been cultivating flood tolerant local rice landraces for over 70 years and pure line selections such as FR13A from Dhalaputia were released in the early 1950s by the Central Rice Research Institute (CRRI), Cuttack, India. These landraces were rediscovered at IRRI in the 1970s, when large-scale screening of the gene bank collection was initiated for improving rice adaptation to less favourable ecosystems (Khush and Coffman, 1977; Ismail and Mackill, 2013). Despite their high tolerance of submergence, these landraces are not suitable for direct use by farmers because they are photosensitive and tall, making them susceptible to lodging, and they have low yield and poor grain quality. However, their discovery made it possible to breed submergence-tolerant varieties and, by the 1990s, tolerant, semi-dwarf and high-yielding lines were developed (Mackill *et al*., 1993). A few of them were released as varieties, but they were not widely grown by farmers because they still possessed some of the undesirable traits of the donors. One of these lines, IR49830-7-1-2-1 with high yield and submergence tolerance was released as Popoul in Cambodia in 1999, though farmers did not adopt it on a large scale.

The availability of molecular markers for genetic studies helped in identifying a major quantitative trait locus (QTL) for submergence tolerance named *SUB1*, on chromosome 9, using a parent that derived its submergence tolerance from FR13A (Xu and Mackill, 1996). *SUB1* controls about 70 percent of the phenotypic variation, and the gene responsible for submergence tolerance was later identified as *SUB1A.* This facilitated the development of a precise marker-assisted backcross system for its transfer into various popular varieties using simple sequence repeat (SSR) markers (Xu *et al*., 2006). *SUB1A* expression is triggered by submergence, causing the plant to remain dormant to conserve energy and also prevent chlorophyll degradation, promoting underwater photosynthesis for additional carbohydrate supply for the submerged plants.

SWARNA, A POPULAR VARIETY IN RAINFED LOWLANDS; THE FIRST TO BE UPGRADED BY SUB1

Swarna (MTU 7029) was developed by Mr V. Ramachandra Rao of the Rice Research Station, Maruteru, Andhra Pradesh, India. It was released in 1982 in Andhra Pradesh, but gradually spread to other parts, particularly eastern and southern India. Currently, it is one of the most popular rice varieties in rainfed lowlands covering about 6 million hectares. Swarna, which means "gold" due to the golden colour of its panicle, became popular because of its high yield, adaptation to low input, moderate tolerance to various stresses, and good grain and eating qualities. The bond between farmers and Swarna in the parlance of agricultural scientists seems to be eternal. The past three-decade long research failed to develop a variety that could effectively replace it despite its sensitivity to flooding. The popularity of Swarna provided an opportunity to use it to dispatch important genes - like *SUB1* - to farmers.

In 2003, IRRI initiated the transfer of the *SUB1* locus into mega-varieties popular in Asia (Neeraja *et al*., 2007; Septiningsih *et al*., 2009). Swarna was crossed to IR49830-7-1-2-1, and the F1 was backcrossed twice to Swarna, and in each backcross a large population was generated and screened with markers to ensure the transfer of the *SUB1* QTL while also recovering the maximum background of Swarna. Through this process of marker-assisted backcrossing, a plant that has the *SUB1* gene but almost all the genome of Swarna was selected and used to produce seeds. Subsequent evaluation of the progeny showed that they are phenotypically identical to Swarna, except that the dark-coloured hulls of Swarna became light or straw-coloured as a result of a gene for hull colour closely linked to *SUB1*. The change of the hull colour is viewed favourably to distinguish the seed of Swarna from Swarna-Sub1. This was completed in 2005 and the new line was named Swarna-Sub1 (Neeraja *et al*., 2007; Mackill *et al*., 2012). Later on, *SUB1* was introgressed into seven other popular varieties from South and Southeast Asia (Septiningsih *et al*., 2009; Mackill *et al*., 2012; Ismail *et al*., 2013). All of them performed typically well under controlled submergence and in farmers' fields while the original varieties showed high mortality when submerged for over one week. Furthermore, introduction of *SUB1* into these varieties did not affect their yield or grain quality (Singh *et al*., 2009; 2011).

FIELD PERFORMANCE OF SWARNA-SUB1 AND ITS COMMERCIALIZATION IN INDIA

With the development of Swarna-Sub1, a long-awaited dream of the Indian farmers in flood-affected areas came true. IRRI sent 200g of seed of Swarna-Sub1 to CRRI in late 2005 which was multiplied in 2006 and shared with other research institutions in India. During 2007 to 2009, a large number of on-station and on-farm field trials were conducted at different locations, evaluating Swarna-Sub1, Swarna and local varieties. In areas where no floods were encountered, the grain yields of Swarna-Sub1 and Swarna were basically the same, indicating that *SUB1* has no effects under control conditions, and provided evidence that Swarna can safely be replaced by the 'improved Swarna-Sub1' to reap the benefit of its submergence tolerance during flood years.

In trials where floods occur either deliberately on stations, or naturally in farmers' fields, survival of Swarna-Sub1 was substantially higher than Swarna. Swarna-Sub1 also recovers faster and generates numerous early tillers that produce fertile panicles resulting in higher yields. The wet seasons (WS) of 2007, 2008 and 2011 were real tests for this variety as most fields experienced flooding, in some cases two to three times during the season. In all trials, Swarna and other varieties either died or produced low yields, while Swarna-Sub1 produced 1.0 to 3.0 t/ha higher than Swarna. In one of the first trials conducted at 32 sites in farmers' fields in Uttar Pradesh, during the WS of 2008, the yields of Swarna and Swarna-Sub1 were similar, at about 5.5 t/ha at sites that did not experience submergence. However, in 24 of these fields submergence occurred for over five days and the average yield of Swarna-Sub1 was 3.98 t/ha compared with 2.68 t/ha for Swarna. The advantage of Swarna-Sub1 over Swarna increased with the length of the submergence.

Similar results were witnessed in trials conducted in thousands of farmers' fields in India, Bangladesh and Nepal between 2008 and 2012. In the WS of 2011, Odisha was badly flooded, causing heavy crop losses and fields that experienced submergence for four to eight days showed losses of 20-70 percent in non-Sub1 varieties, but Swarna-Sub1 showed losses of only 5-9 percent. Numerous additional stories were documented from farmers conducting similar trials (Mackill *et al*., 2012; Ismail *et al*., 2013; www.strasa.org). Persuaded by these encouraging results, both CRRI and the Narendra Dev University of Agriculture and Technology (NDUAT) released Swarna-Sub1 for commercial cultivation in India in August 2009.

Swarna-Sub1 (left) survived 13 days of floods while Swarna (right) did not. Photo taken in Barabanki, Uttar Pradesh in 2012, 50 days after the flood receded

SUB1 IS EFFECTIVE AT VEGETATIVE AND EARLY REPRODUCTIVE STAGES, EVEN AFTER FEW DAYS OF FLOODS

SUB1 is effective at all growth stages from seedling to about a week before flowering (Ismail *et al*., 2013). With early flood damage, farmers usually re-transplant their fields using aged seedlings of local varieties, but this is costly and in some cases not possible as water accumulates fast in the field. In 2008, some fields in Odisha experienced severe floods during panicle initiation (PI) for 12-17 days, and the yield of Swarna-Sub1 was 2.9 to 3.2 t/ha, while surviving Swarna plants did not flower. Similarly, in Uttar Pradesh in 2011, flooding occurred for about one week during PI and in one field Swarna-Sub1 produced 4.75 t/ha and Swarna only 1.76 t/ha. This wide adaptability of Sub1 varieties is important because flooding has become more erratic in recent years. *SUB1* was also effective even after short floods of 2-4 days. Mr Jaipan Parida, a farmer in Dekhta village in Odisha, grew Swarna and Swarna-Sub1 in adjacent fields, and both varieties survived submergence of 4-5 days, but Swarna-Sub1 produced 1.0 t/ha more than Swarna. Similar results were seen in numerous fields and also under controlled floods, indicating that replacing Swarna with Swarna-Sub1 would be useful, even in those areas that experience submergence for less than a week.

PARTNERSHIP AND SUPPORT OF VARIOUS STAKEHOLDERS

The consistent performance of Swarna-Sub1 in farmers' fields resulted in its spread at an unprecedented pace. This is attributed to several factors: (i) the choice of Swarna, the most popular variety in rainfed lowlands, (ii) consistent performance in farmers' fields when flash floods occur for various durations, (iii) similarity to Swarna in all agronomic and grain-quality attributes, and (iv) absence of a yield penalty in non-flood years.

In October 2007, IRRI launched a project titled Stress-Tolerant Rice for Africa and South Asia (STRASA) supported by the Bill and Melinda Gates Foundation. The project built an extensive network of partners from public and private sectors, NGOs and farmers' organizations. STRASA also established linkages with several national initiatives supporting seed production and dissemination. Significant support was also provided by the Government of India through poverty alleviation and climate change programmes, including the "National Food Security Mission", and "Bringing Green Revolution to Eastern India", along with other programmes of state governments. These initiatives identified submergence-tolerant rice varieties as the major technology for promotion.

Various activities were supported to produce seeds and create sufficient demand from seed producers and farmers. Through the support of the state government of Uttar Pradesh, NDUAT produced over 180 tons of the seed during the WS of 2009, the year when Swarna-Sub1 was released, and this provided an impetus for rapid dissemination throughout India – a departure from the norm when only limited quantity of seed of a variety is available at the time of its release, considerably slowing the dissemination process. The state governments of Uttar Pradesh, Bihar, Odisha and West Bengal initiated programmes for multiplication and dissemination of Swarna-Sub1 seed to cover about 1 million ha in each state over three years. These states are also promoting Swarna-Sub1 through other programmes such as seed villages, subsidized seed schemes and seed minikits, all with the purpose of replacing Swarna with Swarna-Sub1 and bringing it to flood-prone areas where it had not previously been possible to grow modern varieties. Approximately 38 000 tons of Swarna-Sub1 seed was produced in the WS of 2011, reaching over 3 million farmers, and covering about 1.1 million ha of rice during the WS of 2012 (Table 1). Apparently, this success was attributed to the catalytic role played by the STRASA project for mustering strong support and commitment from national systems for varietal release, seed policy issues and the vast network of partners engaged in outscaling to reach large numbers of farmers in a relatively short time.

Table 1. Seed multiplication and dissemination of Swarna-Sub1 in India (as of July 2012)

YEAR	No. OF PARTNERS ENGAGED	QUANTITY OF SEED PRODUCED (T)*	ESTIMATES OF AREA COVERED (HA)**	NUMBER OF FARMERS REACHED
2007	21	3		
2008	45	12.5	86	700
2009	100	1 000	357	6 000
2010	120	9 800	28 571	125 000
2011	131	38 126	280 000	1 310 000
2012	140		1 089 314	3 177 167

*Includes both formal and informal (farmer-to-farmer) sectors;
** Estimated based on seed rate of 35 kg/ha in India.

Modified from Ismail *et al*. (2013)

TARGETED DISSEMINATION OF SWARNA-SUB1

Several strategies were followed for the targeted dissemination of Sub1 varieties to meet desired outcomes and impacts:

- Identifying areas affected by flash floods using remote sensing, geographic information systems (GIS) and ground information. Lists of affected villages were provided to state governments for seed distribution.
- The organization of large demonstration plots in areas frequently affected by flash floods as an exhibit to farmers and to produce sufficient seeds for distribution.
- The introduction of minikit programmes; 5 kg seed packages distributed once to 5-10 farmers in each village, then their spread monitored within villages and between neighbouring villages.
- The prioritization of seed distribution, with preference being given first to villages that are severely affected by flash floods, then to less flood-prone villages and, ultimately, the replacement of Swarna with the improved Sub1 version in both rainfed and irrigated lowlands.
- Promoting Sub1 varieties in areas where modern varieties could not be grown before because of their sensitivity to submergence.

In summary, submergence-tolerant varieties provided opportunities for improving and stabilizing yields in flash flood-affected areas, significantly contributing to national food security. Through the consolidated efforts of STRASA and partners, and thanks to enormous support from the Indian Government, Swarna-Sub1 has now reached a large number of farmers. Key elements to this success include the choice of the variety, the strong financial and policy support and commitment from the national system in facilitating early release, promotion and provision of sufficient, high quality seeds and knowledge, and targeted dissemination. These varieties also provided additional opportunities for enhancing annual productivity through use of input and adjusting cropping patterns. Sub1 varieties and varieties tolerant of drought and salt stress are currently becoming available through joint efforts of IRRI and national agricultural research and extension systems (NARES) partners, and these varieties are expected to transform agriculture in the less favourable rainfed areas.

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