



BIOTECHNOLOGIES AT WORK FOR SMALLHOLDERS: CASE STUDIES FROM DEVELOPING COUNTRIES IN CROPS, LIVESTOCK AND FISH



OCCASIONAL PAPERS ON **INNOVATION IN FAMILY FARMING**

**BIOTECHNOLOGIES AT WORK
FOR SMALLHOLDERS:
CASE STUDIES FROM
DEVELOPING COUNTRIES IN
CROPS, LIVESTOCK AND FISH**

Edited by

J. Ruane, J.D. Dargie, C. Mba, P. Boettcher, H.P.S. Makkar, D.M. Bartley and A. Sonnino

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ABBREVIATIONS AND ACRONYMS

ABDC-10	FAO international technical conference on Agricultural Biotechnologies in Developing Countries
ACIAR	Australian Centre for International Agricultural Research
AGE	Joint FAO/IAEA Programme for Nuclear Techniques in Food and Agriculture
AI	Artificial insemination
AICPMIP	All-India Coordinated Pearl Millet Improvement Project
AR4D	Agricultural research for development
AW-IPM	Area-wide integrated pest management
BBrMV	Banana bract mosaic virus
BBSRC	Biotechnology and Biological Sciences Research Council (in the United Kingdom)
BBTV	Banana bunchy top virus
BMP	Better management practice
BRAC	Bangladesh Rural Advancement Committee
CAI	Cervical artificial insemination
CCSHAU	Chaudhary Charan Singh Haryana Agricultural University (in India)
CDVF	Community-based Dairy Veterinary Foundation (in Bangladesh)
CGIAR	Consultative Group on International Agricultural Research
CIAT	International Centre for Tropical Agriculture
CMD	Cassava mosaic disease
CMV	Cucumber mosaic virus
CNPq/REPENSA	National Research Networks in Agrobiodiversity and Agricultural Sustainability programme (in Brazil)
COOASGO	Cooperativa Agropecuária São Gabriel do Oeste Ltda (in Brazil)
CRRRI	Central Rice Research Institute (in India)
CS	Case study
CSIR-CRI	Council for Scientific and Industrial Research-Crops Research Institute (in Ghana)
DFID	Department for International Development (United Kingdom)
DM	Downy mildew
ELISA	Enzyme-linked immunosorbent assay
EMBRAPA	Brazilian Agricultural Research Corporation
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FHIA	Fundacion Hundurena de Investigacion Agricola
FIDAR	Fundación para la Investigación y Desarrollo Agrícola (in Colombia)
FSD	Frog skin disease
GIS	Geographic information systems

HPV	Hepatopancreatic parvovirus
IAEA	International Atomic Energy Agency
IBERS	Institute of Biological, Environmental and Rural Sciences (in United Kingdom)
ICAR	Indian Council of Agricultural Research
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IDRC	International Development Research Centre (in Canada)
IFAD	International Fund for Agricultural Development
IITA	International Institute of Tropical Agriculture
INIVIT	Instituto de Investigaciones de Viandas Tropicales (in Cuba)
INTA	Instituto Nacional de Tecnología Agropecuaria (in Argentina)
IPR	Intellectual property rights
IRRI	International Rice Research Institute
LAI	Laparoscopic artificial insemination
LAMP	Loop-mediated isothermal amplification
LANAVET	Laboratoire National Veterinaire (in Cameroon)
MABC	Marker-assisted backcrossing
MAPA	Ministry of Agriculture, Livestock and Food Supply (in Brazil)
MAS	Marker-assisted selection
MBV	Monodon baculovirus
MCTI	Ministry of Science, Technology and Innovation (in Brazil)
MINEPIA	Ministry of Livestock, Fisheries and Animal Industries (in Cameroon)
MPEDA	Marine Products Export Development Authority (in India)
MrNV	Macrobrachium rosenbergii nodavirus
MTA	Material transfer agreement
NACA	Network of Aquaculture Centres in Asia-Pacific
NaCSA	National Center for Sustainable Aquaculture (in India)
NARES	National agricultural research and extension systems
NARI	Nimbkar Agricultural Research Institute (in India)
NARS	National agricultural research systems
NDUAT	Narendra Dev University of Agriculture and Technology (in India)
NGO	Non-governmental organization
NRCRI	National Root Crop Research Institute (in Nigeria)
OIE	World Organisation for Animal Health
PATTEC	Pan African Tsetse and Trypanosomiasis Eradication Campaign
PCR	Polymerase chain reaction
PL	Postlarvae

PPR	Peste des petits ruminants
PVS	Productivity veterinary service
QTL	Quantitative trait locus
R&D	Research and development
RFLP	Restriction fragment length polymorphism
RT-PCR	Reverse transcriptase PCR
SIT	Sterile insect technique
SPF	Specific pathogen-free
SPIA	CGIAR Standing Panel on Impact Assessment
SSA	Sub-Saharan Africa
SSR	Simple sequence repeat
STRASA	Stress-Tolerant Rice for Africa and South Asia
USAID	United States Agency for International Development
WS	Wet season
WSSV	White spot syndrome virus

CHAPTER 1

INTRODUCTION

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Strong economic growth over the past 20 years has reduced by half the number of people in developing regions living in extreme poverty (UN, 2012). The agricultural achievements of past decades have been impressive. Even as the earth's human population increased by roughly 1 billion, the prevalence of hunger dropped to less than 15 percent, and people have increasingly been consuming more nutritious foods. Nevertheless, 842 million people were estimated to be suffering from chronic hunger in 2011–13, regularly not getting enough food to conduct an active life (FAO, IFAD and WFP, 2013). Most of these people live in rural areas and depend for food on small-scale farming and marketing systems. The projected increase in the global population to 9 billion and, with this, the expected 60 percent increase in the demand for food by 2050 leaves no room for doubt that individual countries and the international community need to make an extra effort to assist the poorest and most marginalized to secure their right to adequate food (Place *et al.*, 2013).

The challenges faced in tackling and eliminating food insecurity and malnutrition – and doing so sustainably and equitably – are substantial. For example, rural populations are becoming relatively smaller as people move to cities. Farmers, fishers and forest-dependent people are facing new risks from climate change, disease and the overuse of renewable natural resources. The demand for biofuels is changing the way land is being used, and the current financial crisis and recent spikes and volatility in food prices are of increasing concern worldwide. These and other threats to the global food and agriculture landscape are certainly worrying but, while both the causes of and the solutions to hunger and malnutrition are often complex and varied, they are not insurmountable.

The key lies in empowering the millions of smallholder producers and landless workers who form the backbone of rural economies in most developing countries to grow their incomes and improve their livelihoods by raising agricultural productivity and engaging in

markets. In effect, investments in agricultural development can be used as the catalyst for encouraging broad-based rural development and provide the basis for meeting hunger and wider sustainable development objectives such as reducing poverty, food and nutritional insecurity and environmental degradation. That such investments have paid off handsomely in the past (and continue to do so) is convincingly illustrated by the publication *Millions fed: Proven successes in agricultural development* (IFPRI, 2009). Of all the ingredients of success, arguably the most important, and certainly the most common, has been sustained public investment both in the research that developed technologies and knowledge for increasing land, water and labour productivity and in the extension and community services that promoted their diffusion.

Of course, the realities of producing, marketing and trading in food and agricultural products are more complex nowadays, which creates both risks and opportunities for farmers and consumers alike. Even so, the science-based technologies and knowledge now available to tackle these ever-changing challenges are also rapidly evolving. New and smarter science-based products, processes and ways of working continue to come on stream. These offer real opportunities for contributing to the elimination of hunger and malnutrition, provided that they are appropriately integrated with longer established approaches and traditional knowledge and skills, and that they are also underpinned by institutional and financial arrangements that recognize the vital role of smallholder production and marketing systems. The need to make such changes to improve people's lives is now more pressing than ever.

Agricultural biotechnologies are prominent among the suite of innovations available to producers through the national, regional and international research systems, extension services, ministries, civil society organizations and private sector companies that support them. FAO has long recognized the considerable potential of the tools provided by both new and older biotechnologies to promote sustainable agrifood development, but is also aware of the concerns surrounding some applications.

To provide a forum for the exchange and subsequent dissemination of objective science-based information about these technologies, it organized an international technical conference in Mexico in collaboration with global partners (FAO, 2011). Entitled "Agricultural biotechnologies in developing countries: Options and opportunities in crops, forestry, livestock, fisheries and agro-industry to face the challenges of food insecurity and climate change (ABDC-10)", this Conference brought together about 300 policy-makers, scientists and representatives of intergovernmental and international non-governmental organizations, including delegations from 42 FAO Member Nations. Plenary presentations covered the

subject rigorously in all its dimensions, and delegates were provided with peer-reviewed background documents outlining the current status and options for using biotechnologies within all the major food and agricultural sectors, as well as the related policy issues. Coupled with presentations of case studies of successful applications of biotechnologies in developing countries, the rich and wide-ranging discussions during subsequent sector-specific, cross-sectoral and regional sessions offered delegates ample opportunity to reflect on lessons learned and consider options for developing countries as well as priorities for action by the international community.

One of the key messages to come out of the Conference was that, although biotechnologies are being applied to an increasing extent to alleviate hunger and poverty, adapt to climate change and maintain the natural resource base, they have still not been widely applied in many developing countries, and have not sufficiently benefited smallholder farmers and poor consumers. It is outside the scope of this publication to attempt even to summarize the great variety of factors responsible for this state of affairs. Nor do we have space here for a description of all the agricultural biotechnologies that are available for application on-farm or in laboratories, directly supporting farmers and national agricultural development and global “public goods”, e.g. by analysing samples for a crop pest or animal disease. Readers interested in these aspects are referred to FAO (2011) or to Ruane and Sonnino (2011).

Even so, policy-makers and investors in agricultural development need to be able to tell what the most pressing needs in a country are to overcome hunger and malnutrition. They have to prioritize among the many competing demands for limited human and financial resources, and they need hard evidence that biotechnologies can tackle these priority issues. They also want assurances that R&D will have the desired impact when adopted by potential end-beneficiaries or used by service providers. They must ask themselves whether research results will translate into economic benefits for smallholder farmers and consumers, into better nutrition for their children, into lower pesticide use, into less disease etc.

A robust *ex ante* project design is a prerequisite for securing funding for development research and ensuring it achieves the desired outcomes. The project must therefore identify the pathways through which the intended interventions can achieve the desired impact. Together with rigorous priority and needs assessments, impact pathways and associated performance indicators that set out, among other things, what will be achieved, by whom, where and when are key components of a monitoring and evaluation system, which will support feedback and learning. Upon completion of the intervention, the *ex post* impact assessment seeks to establish whether the technological package actually improved rural livelihoods.

Making the link between agricultural biotechnologies and economic, social and environmental outcomes and impacts is neither conceptually nor practically straightforward. Methodological challenges in the economic sphere (see e.g. de Janvry, Dustan and Sadoulet, 2011) become even more complex when measured also with reference to “people benefits”, such as better nutrition or improvements in the biophysical environment. Further, formal impact analysis takes time and requires, among other things, the collection and evaluation both of objective information and of subjective stakeholder assessments at the micro and macro level, as well as substantial levels of funding, computer modelling and wider project management skills. All of these are in short supply in most developing countries. Little wonder, then, that the information base about what biotechnologies work for agricultural development is so narrow.

Inspiration for this publication came from an initial series of five short sector-specific parallel sessions which took place during ABDC-10 (session reports are provided in Chapter 11.3 of FAO [2011]) dealing with case studies of what were generally considered to be “successful applications” of biotechnologies in developing countries. During these sessions, two to three case studies were presented, followed by a facilitated discussion, providing people with an opportunity to evaluate the key factors responsible for the results. This publication attempts to build upon these sessions and to examine in greater detail some of the issues they raised. It does so through the presentation of 19 case studies written by different authors covering the crop, livestock and fisheries/aquaculture sectors (with five to seven studies for each sector). They were chosen after a widely disseminated open call for proposals relating to case studies in which biotechnologies have been applied to serve the needs of smallholders in developing countries.

The publication also endeavours to pull out “lessons learned”, i.e. the obstacles/challenges encountered, what worked and why, while recognizing the many pitfalls in claiming “success” in achieving the ultimate long-term goal of actually reducing hunger, malnutrition or poverty. Indeed, most of the reported successes relate to meaningful outcomes. That is to say, they describe instances in which the technology and related knowledge were used and adopted by smallholder producers, who thereby set in motion a series of changes that ultimately improved their own and other people’s lives. While these case studies may be best described as initial steps towards assessing the benefits of agricultural biotechnologies, it is also hoped that they will provide useful information for policy-makers, potential investors, development specialists and scientists and encourage further investments both in R&D and in the diffusion of these and other biotechnologies.

Several criteria were used for choosing the case studies. First, what constitutes a biotechnology? The definition was taken from the Convention on Biological Diversity and was

also used at ABDC-10, i.e. “any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use”. As such, it covers a broad range of technologies – often referred to variously as “traditional/conventional” or “low-tech” through to “modern” or “advanced” approaches – that are used for a number of different purposes, such as the genetic improvement of plants and animals to increase their yields or efficiency; the characterization and conservation of genetic resources for food and agriculture; plant and animal disease diagnosis; and the production of fermented foods. The publication tried to reflect the breadth of this definition by choosing case studies relating to several different biotechnologies and diverse applications.

Second, what constitutes a case study? Here, it was decided that it must involve the application of the biotechnology (or biotechnologies) in the field, and so should have gone beyond the research or experimental stage. To qualify for inclusion, a case study did not necessarily have to represent a success, defined in terms of “people benefits”. Rather, the aim was to produce a package of case studies that would illustrate the very different realities of applying biotechnologies in developing countries, and consider the many variables such as context, background/history, key problem(s) to be addressed, the specific biotechnology itself and the mode of implementation, the obstacles and challenges encountered, the factors of success (or failure), the impacts and the lessons learned.

Third, what is a smallholder farmer? Here, there was more scope for flexibility since the definition varies from country to country and between agro-ecological zones (FAO, 2004). In favourable areas with high population densities, a smallholder may often cultivate less than one hectare of land, keep two or more cows and/or have a backyard family fish pond of perhaps 200 m², whereas in semi-arid areas they may cultivate 10 ha or more, or manage 10 head of livestock.

Finally, in selecting the case studies, every effort was made to ensure that they came from different regions of the developing world and that, within each sector, a range of applications involving different species was included. The 19 case studies that were finally chosen are illustrated in the following three chapters and cover the crop, livestock and aquaculture/fisheries sectors respectively. They include applications of biotechnologies to overcome biological and technological constraints in order to increase productivity, improve people's livelihoods, tackle diseases and pests, expand market opportunities through diversification and value addition, and conserve some of the unique but threatened genetic resources needed for ensuring the sustainability of smallholder production systems. The final chapter attempts to summarize both the benefits and downsides of the technologies introduced and to recapitulate the lessons learned from technical, policy and institutional perspectives.

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CHAPTER 2

CASE STUDIES IN THE CROP SECTOR



Evaluation of the field performance of banana plants derived from tissue culture
©Karin Nichterlein



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,

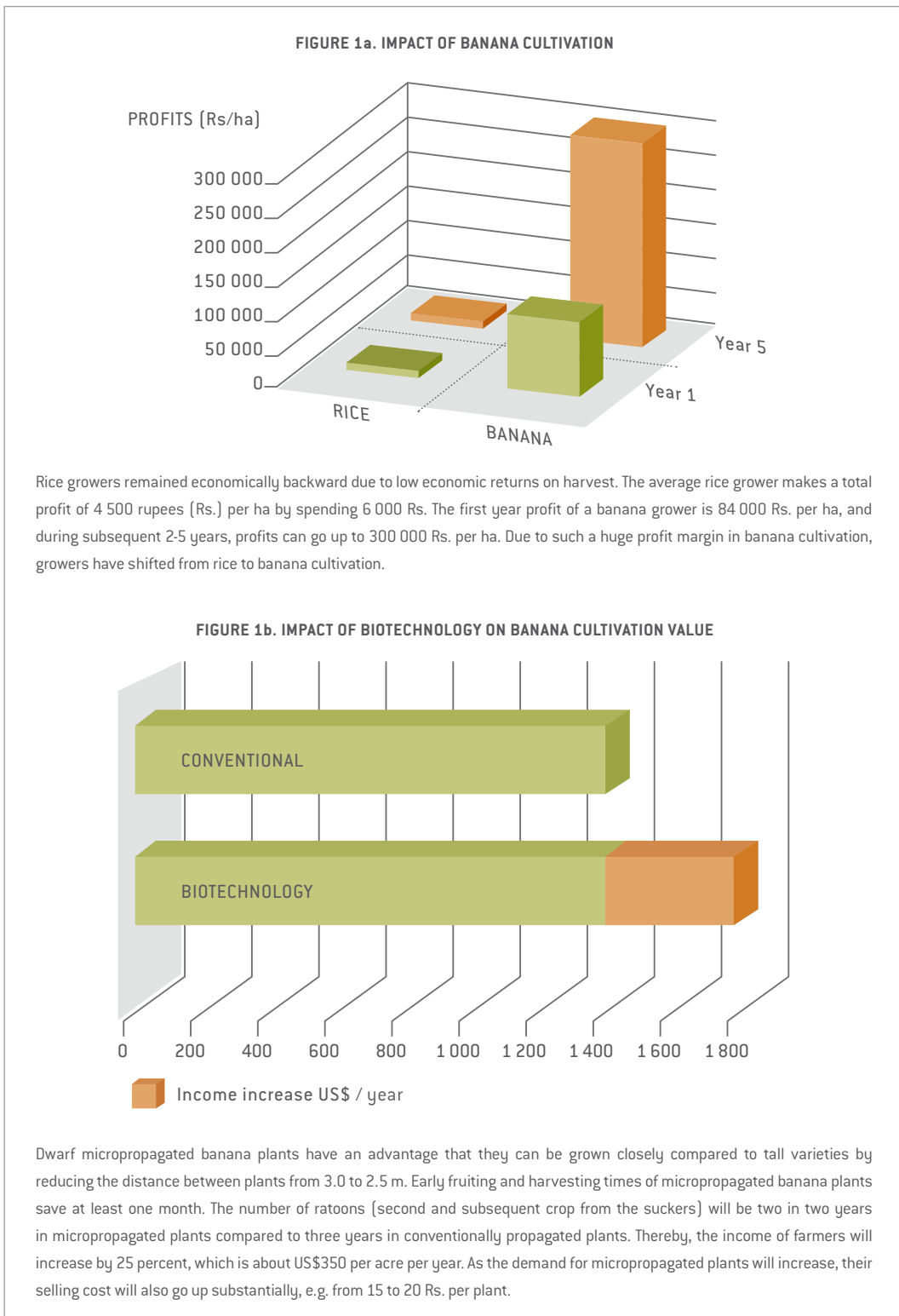
natural and induced diversity, and conservation are equally important for banana improvement. The loss of genetic diversity can have a serious impact on banana cultivation because of lack of new improved cultivated varieties. There is hardly any disease resistant banana variety available to the growers. 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



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

@Karin Nichterlein

Banana plantlets transferred from *in vitro* conditions to potting soil (to slowly adjust to natural conditions)

@Karin Nichterlein

Production of banana derived from micropropagation in the farmer's field

@Karin Nichterlein

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	<ul style="list-style-type: none"> 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
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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.

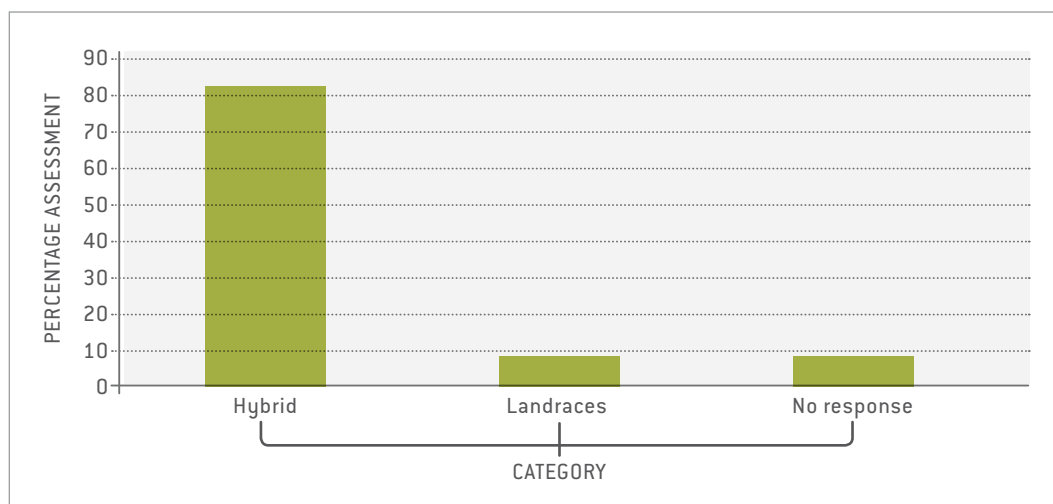
This system reportedly reduced disease pressure in fields so that there were healthier crop stands, which produced increased tuber yields of 12.3 percent and 30 percent for two released sweet potato varieties, Sauti and Faara, respectively (Otoo and Quain, 2001). These two varieties were established 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
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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
<i>In vitro</i> -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 27.8×10^4 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

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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 a 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 Accesoría 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 Piendamó'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
©CIAT/Roosevelt H. Escobar

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
@Sudhanshu Singh

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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CHAPTER 3

CASE STUDIES IN THE LIVESTOCK SECTOR





A Deccani flock with FecB carrier ewes being led for grazing
©NARI /Chanda Nimbkar

CHAPTER 3.1

SUSTAINABLE IMPROVEMENT IN SHEEP PRODUCTIVITY IN INDIA USING THE FECB (BOORoola) MUTATION

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BACKGROUND AND CONTEXT

Sheep-rearing is a traditional source of livelihood for communities in the drought-prone rural areas of India. Together, these livestock keepers tend about 70 million sheep (GOI, 2010). Most of the sheep in India are reared in the states of Andhra Pradesh, Rajasthan, Karnataka, Tamil Nadu and Maharashtra (listed in the order of sheep population size from the largest to the smallest). The major income from rearing Deccani sheep on the Deccan plateau in Maharashtra state is earned from the sale of lambs at 3 to 4 months of age. Lambs are usually sold in groups and butchers purchase them on visual inspection. As a result, a sheep owner's income depends largely on the number of saleable lambs produced per ewe per year. The reproductive rate is therefore important in such a system where lamb production is the primary product of the ewe and usually the main reason for her existence. Most of the costs of maintaining ewes have to be borne by the lamb(s) produced (Wiener, 1988). Only about 2 percent of Deccani ewes have twin lambs (Waghmode, 2007). Shepherds keep breeding rams with ewes and, with good nutrition, ewes will lamb every 9 to 10 months. The sale price of lambs has increased by 10 to 20 percent per year over the last 10 years or more, due to the increasing human population, urbanization and incomes, as well as the increasing gap between demand and supply of meat.

Mr B.V. Nimbkar, the founder of the Nimbkar Agricultural Research Institute (NARI), a non-governmental, non-profit foundation established in 1968 in Phaltan in Satara District of Maharashtra in western India, used to have his own sheep and had observed the sheep-rearing scenario around him for a long time. He was appointed in 1988 by the Government of Maharashtra as the chairman of a "Commission to study the problems of goat and sheep production in Maharashtra". He realized in the course of reading about sheep and goat production worldwide that there were prolific indigenous sheep breeds in many parts of the world and that their owners benefited from having more lambs to sell. He discovered from the renowned Australian sheep geneticist, the late Dr. Helen Newton Turner, about the prolific "Bengal" sheep that had been taken to Australia in 1792 (10 ewes and 2 rams) and in 1793 (another 100 ewes) and that were later identified as the probable source of the *FecB* (Booroola) gene, which has a powerful effect on ovulation rate, to which the fecundity of the Booroola Merino was attributed in the 1980s (Davis *et al.*, 1982). Mr Nimbkar found out about the prolific Garole (Bengal) sheep in Sundarban in West Bengal state in north-eastern India in 1992, and procured 32 ewes and 12 rams in 1993 and 1994, bringing them to Phaltan over 1 500 km by train.

Breeding of Deccani ewes of the Lonand type with Garole rams (by artificial insemination because of the small size of the Garole compared with the Deccani) began at NARI in 1996 to increase the prolificacy of the Deccani. After the *FecB* mutation was identified and a DNA test for its presence developed (Wilson *et al.*, 2001), it was confirmed that the prolificacy of the

Garole was indeed because of the *FecB* gene (Davis *et al.*, 2002). The partially dominant mode of inheritance of *FecB* meant that after the first cross to introduce the gene, the prolificacy could be retained in the backcrosses by selecting only the carriers for breeding. The backcrossing was necessary to ensure the *FecB* carrier animals looked like the Deccani and had its larger body size, hardiness, adaptation to harsh conditions and good mothering ability.

BIOTECHNOLOGY APPLIED

The DNA test to detect the *FecB* mutation is based on the polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) procedure (Wilson *et al.*, 2001). The technical capacity to perform the test in India was initially established at the National Chemical Laboratory, and from 2009 at NARI with technical guidance from Australian scientists under a project funded by the Australian Centre for International Agricultural Research (ACIAR). The PCR-RFLP assay was an essential prerequisite for the success of the breeding programme at NARI to introgress the *FecB* mutation into the local sheep breed. This is because:

1. the phenotype of *FecB* carriers (increased number of ovulations and lambs) cannot be measured in males or before the age of puberty in females;
2. without the DNA test, laparoscopic counting of ovulations (which requires skill and is tedious, labour-intensive and invasive) is the only method of assessing the *FecB* genotype of post-pubertal females;
3. the phenotype is not completely associated with genotype in females (i.e. a female that bears two lambs is more likely to carry the *FecB* mutation, but not necessarily so, and carrier ewes do not have twins at every lambing).

Now a few drops of blood of newborn lambs from NARI's or shepherds' flocks are simply collected on Whatman FTA™ classic cards and their ear tag numbers are written on the cards. DNA is isolated from these blood samples, and the PCR-RFLP *FecB* test is carried out at NARI's molecular biology laboratory established under a project funded by the Department of Biotechnology, Government of India. If the protocols are followed strictly, about 90 lambs can be genotyped in less than two days with close to 100 percent accuracy. This facilitates selection and culling decisions on lambs at a very young age.

Rams and ewes carrying the favourable *FecB* allele, but looking mostly like Deccani sheep, have been introduced into flocks of smallholders. The ewes introduced were mostly B+ (carrying one copy of the favourable B allele for prolificacy and one copy of the normal + allele) while about half the introduced rams were BB and half B+. One copy of the *FecB* gene increases the average litter size of Deccani sheep from 1.0 lamb per lambing to 1.5 lambs per lambing (i.e. 15 instead of 10 lambs per 10 ewes lambing). Better nutrition of ewes at the time of breeding can increase



from left to right

Mr Pisal in 2012 with the breeding ram carrying one copy of the *FecB* gene born in his flock

@NARI /Chanda Nimbkar

Mr Kavitke (middle) with a ewe carrying two copies of the *FecB* gene and its 3-weeks old triplet lambs

@NARI /Chanda Nimbkar

Mr and Mrs Pisal of Bhadali with one of their NARI Suwarna ewes in 2004

@NARI /Chanda Nimbkar

the number of lambs born per ewe up to 1.6. Better nutrition and management of ewes and their lambs ensures the survival of most of those lambs, guaranteeing a higher income to the owner, after deducting the cost of the supplementary feed given to pregnant and lambing ewes and their lambs. The average litter size of ewes with two copies of the B allele is about 1.8 in the NARI flock, but there are very few BB ewes in smallholders' flocks so far.

IMPLEMENTATION OF THE BIOTECHNOLOGY

Rams carrying the *FecB* gene were introduced into 26 local sheep owners' flocks from 2003 onwards. Sixty *FecB* carrier (B+) ewes and 60 non-carrier ewes were introduced. The sheep owners were selected on the basis of their contact with NARI over the previous few years through NARI's field research and extension activities and their willingness to participate in the project (Prior *et al.*, 2009). All animals in the participating flocks were ear tagged; all lamb births, sales and deaths and weights of all animals every two months were recorded. Another 94 *FecB* carrier ewes were purchased from NARI by 12 local smallholders in January 2010 with bank loans. *FecB*-carrier breeding rams were sent to these smallholder flocks for free by NARI. Two of these smallholders had *FecB* carrier ewes in their flocks since 2003. One of these two, Mr Dattatray

Sopan Pisal, purchased 13 more *FecB* carrier ewes from NARI again in February 2012. The current breeding ram in Mr Pisal's flock was born in the flock and genotyped at NARI to confirm its *FecB* carrier status. Mr Pisal has sold more than 50 *FecB* carrier young ewes for breeding over the last five years to other smallholders around him. NARI has now officially designated Mr Pisal's flock as a multiplier flock. At least two people have been inspired by Mr Pisal's success to take up sheep-rearing using *FecB* carrier ewes purchased from his flock (see Box for an example).

Mr Shivaji Kavitke of Kothale village in Malshiras taluka of Solapur district in Maharashtra state used to work in Mumbai, the capital of Maharashtra, as a labourer at the port trust. He retired in 2000 and returned to his village and farm. He belongs to the Dhangar (shepherd) community and decided, in 2012, to start rearing sheep again – the family's traditional occupation. He purchased 14 non-pregnant ewes carrying one copy of the *FecB* allele (B+ genotype) and 4 ewes carrying two copies (BB), from NARI in February 2012. He also brought a NARI B+ breeding ram from the flock of another shepherd participating in NARI's project and released him in his own flock. Ten months after the purchase, (we visited him on 22 December 2012), these ewes had 29 lambs - 9 lambs from the 4 BB ewes (2.25 per ewe) and 20 lambs from the 14 B+ ewes (1.43 per ewe). Twenty lambs were about 3 months of age and nine were less than one month old. There was no mortality among lambs or ewes. The 3-month weight of single born lambs was about 14.5 kg while the combined weight of twin lambs was 21 to 24 kg. Ewes that had lambed 3 or more months before, had exhibited oestrus and been mated.

Table 1: Mr Kavitke's expenditure and earned and expected income from the first lamb crop of *FecB* carrier ewes purchased in February 2012 (based on records kept by Mr Kavitke's nephew)

EXPENDITURE (US\$) ^a		INCOME (US\$)	
Purchase of <i>FecB</i> carrier ewes from NARI: 18 ewes at Rs.2 300 per ewe ^b	755.5	Sale of two lambs	73.0
Purchase of maize grain	73.9	Sale of sheep manure ^c	56.6
Chain link fencing	45.6	Expected sale price of 18 lambs to be sold in Jan. 2013	821.2
Tarpaulin to protect sheep from rain	13.9	Expected sale price of 9 lambs to be sold in Mar. 2013	410.6
Vet. charges and medicines	28.0		
Total expenditure	916.9	Total income	1 361.4

a US\$1 = Rs.54.8

b The ewes sold by NARI were 'second category' and some were >4 years old. They were sold at a subsidized price.

c The remaining manure was spread on Mr Kavitke's own land.

IMPACT

Table 2 shows that *FecB* carrier ewes had 27-46 percent higher productivity in terms of 3-month old lambs produced than non-carrier ewes in smallholders' flocks.

Table 2: Live litter size of *FecB* carrier and non-carrier ewes introduced in 2003 and 2010 and born in smallholder sheep-owners' flocks within a 25-km radius of Phaltan

TRAIT	No. OF FLOCKS	No. OF EWES		YEAR	EWE'S <i>FecB</i> GENOTYPE	
		++	B+		++	B+
Live litter size at birth per ewe lambing with at least one live lamb ^a	22	959	187	2004-08	1.03 (2 406)	1.42 (325)
Live litter size at 3 months per ewe lambing with at least one live lamb ^a					0.95	1.21
Live litter size at birth per ewe lambing including stillbirths ^b	9	244	114	2010-11	1.03 (482)	1.52 (244)
Live litter size at 3 months per ewe lambing including stillbirths ^b					0.98	1.43

Figures in brackets are the number of records.

^a Nimbkar *et al.* (2009a)

^b Nimbkar *et al.* (2013)

During the period from 2006 to 2008, the gross margins per *FecB*-carrier ewe in Mr Pisal's flock (with 8, 41 and 50 *FecB* carrier ewes in the three years, respectively) were 37-50 percent higher than for non-carrier ++ ewes (Nimbkar *et al.*, 2009a). Table 3 shows the income per ewe per year (for ewes born between 2004 and 2008) in the same flock from January 2010 to January 2012. It was Rs.1 177 per ewe for 22 non-carrier ewes and Rs.1 869 per ewe for *FecB* carrier ewes (3 BB and 31 B+ ewes).

Table 3: Income from *FecB* carrier (B+ and BB) and non-carrier (++) ewes in Mr Pisal's flock from January 2010 to January 2012 (unpublished data)

<i>FecB</i> GENOTYPE OF EWE	No. OF EWES	No. OF LAMBINGS (INCLUDING ABORTIONS)	LAMBS BORN ALIVE	LAMBS SOLD	WEIGHTED AVERAGE SALE PRICE PER LAMB (Rs.)	AVERAGE INCOME PER EWE PER YEAR*
++	22	33	32	19	1 850	1 177
B+ and BB	31 and 3	71	98	52	1 412	1 869

* Average income per ewe per year = $\frac{\text{No. of lambs alive at 3 months} \times \text{weighted average price of lambs}}{2 \text{ years} \times \text{No. of ewes}}$

OBSTACLES / CHALLENGES ENCOUNTERED

1. Winning the confidence of deeply traditional sheep owners was a challenge. The idea of introducing twinning was novel to them and they were not so receptive to it at first; one of the reasons being they thought it involved greater risk.

2. Two-year time lag between ram introduction and twinning of ewes: Capacity-building was needed to explain to some sheep owners that it was the daughters of the *FecB*-carrier rams that would have twin lambs and not the ewes that were mated to them. Deccani ewes lamb for the first time at the age of about 18 months. If the twinning technology is introduced only through rams, there is a long time lag between its introduction in a flock and benefits in the form of twin lambs, and a further lag of three months before the lambs are sold and monetary benefits realized.

3. Carrying the *FecB* mutation does not guarantee twins: This biological phenomenon has impeded the acceptance of the technology. Good nutrition of the ewes at the time of breeding leads to a higher incidence of twinning among *FecB*-carrier ewes but the mechanism involved is not fully understood. Ovulation rate and litter size are probably influenced by other genes and by non-genetic factors, so these traits vary among ewes with the same genotype and among successive parturitions of the same ewe. *FecB*-carrier ewes may have singles, twins or triplets at different lambings and some ewes have twins more consistently than others. Sheep flock owners find it difficult to understand this uncertainty.

4. Sheep owners' changed preference for Madgyal rams instead of Deccani or Madgyal cross rams: Madgyal is a breed from Sangli district in Southern Maharashtra and adjoining areas of Karnataka state that is taller and larger than the Deccani. Madgyal cross lambs are therefore almost twice as large as Deccani lambs of the same age and grow much faster. From 1994, when the project began, to 2003 when the dissemination started, the Deccani sheep owners' preference for breeding rams changed markedly from using Deccani or Madgyal X Deccani rams to pure Madgyal rams, while the *FecB*-carrier rams NARI was disseminating were mostly 75 percent Deccani and 25 percent Garole. Sheep owners preferred facial features such as a narrow forehead and a Roman nose, but the *FecB*-carrier rams have the shorter stature and wide forehead of the Garole. NARI consequently started using Madgyal rams for breeding in the nucleus flock in December 2006. Madgyal rams were mated to *FecB*-carrier ewes to produce B+ 50 percent Madgyal progeny. However, this meant a reduction in the frequency of the B allele, since Madgyal rams were non-carriers of *FecB*. Inter-se mating and backcrossing were used to produce 75 percent Madgyal B+ and BB rams. NARI began to offer sheep owners B+ rams

of a Madgyal-like phenotype from 2009, and BB rams similar in appearance to Madgyal from 2012. The larger size of Madgyal rams did not lead to any lambing difficulties and improved the survival of crossbred lambs. It is, however, taking time to change the earlier negative perceptions of *FecB*-carrier rams in the minds of sheep owners.

FACTORS IMPORTANT FOR SUCCESS/FAILURE

1. Pressure for early dissemination of *FecB* carrier rams and ewes: In an “introgression” programme such as this one, where it is desired to introduce only a single gene influencing a desired characteristic (i.e. litter size in this case) from a particular donor breed into a recipient breed, at least four generations of backcrossing with the recipient breed are recommended prior to dissemination (van der Werf, 2009). The aim of the backcrossing is to recover the genome of the recipient breed and to ensure that the introduced gene carrier animals look and perform like the recipient rather than the gene-donor breed. In this programme, there was pressure to start disseminating gene-carrier animals after only two generations of backcrossing (i.e. animals having 25 percent or more Garole genetics), in order to determine whether the encouraging results observed in the NARI flocks would also be apparent in the traditional smallholder sheep-owning environment. Because of the high Garole proportion, the animals had some undesirable attributes compared with the Deccani, such as a short stature, horns, an undesirable appearance and poor mothering ability. The sheep owners’ changed preference for the Madgyal breed compounded the problem, as explained above.

2. Choosing the right sheep owners/flocks: The “twinning technology” should be introduced only to sheep owners who practice good animal husbandry and are proficient at managing the commercial aspects such as judging the right time for selling lambs and obtaining the maximum prices. We found that some sheep owners are skilled at getting ewes that had aborted or had stillborn lambs to accept other ewes’ lambs. Such skill at promoting the cross-fostering of lambs is one of the keys to successfully managing an increasing proportion of twins and triplets. The technology is also more likely to succeed with farmers who can take advantage of increased lamb numbers, for example those who have irrigation to grow nutritious fodder, or enjoy access to extra labour, or those who are more settled and less nomadic (Prior *et al.*, 2009). The ewes carrying twin foetuses also need to be given a small quantity of supplementary feed from about a month to 45 days before lambing until about 3 months after lambing. Maize grain is a good and reasonably priced nutritional supplement and has also become readily available in recent years. Farmers who have irrigation have started to grow it commercially because of the high yields of the new hybrids.

3. Accompanying support in key areas such as veterinary health: NARI's extension staff members have observed that sheep owners in whose flocks the twinning technology was introduced highly valued the veterinary health support they received in managing their flocks (Prior *et al.*, 2009). Such support enhances the chances of success of the technology by decreasing ewe and lamb mortality. NARI has made this intervention more sustainable by training sheep owners to vaccinate their flocks and treat minor infections in their animals. Women and school or college-educated young members of the family were found to be willing and effective learners.

ISSUES TO BE CONSIDERED FOR MORE WIDESPREAD APPLICATION OF THE TECHNOLOGY

Selected competitive animals with more than 90 percent Deccani or 50 percent Madgyal genetics and carrying the desired *FecB* allele are now available from NARI. Using these, introgression into a larger number of appropriate flocks in suitable areas of the Maharashtra and Karnataka states could start straight away. It is necessary, however, to popularize the technology through publicity and by bringing sheep owners to visit NARI and smallholders that have reasonable numbers of *FecB*-carrier ewes. The owners of flocks where introgression is started should be given training, problem-solving and other kinds of support, at least for the first five years.

In other states of India where distinctly different breeds are reared, there would have to be three to four generations of backcrossing so that the *FecB*-carrier animals would have a similar phenotype as the original breed. The institution that would carry out the introgression should, therefore, have the appropriate infrastructure to maintain a large sheep flock of several hundred ewes at least, and to carry out backcrossing, data recording and genetic evaluation. The same institution, or else a separate one with a network and regular extension and training activities among sheep owners, could carry out the dissemination (Nimbkar *et al.*, 2009b). Smallholder sheep owners are likely to welcome introgression if the *FecB*-carrier animals are phenotypically superior and if they find the increased lambing rate profitable.

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A herd of Namaqua Afrikaner ewes and lambs at the Carnarvon Experimental station
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CHAPTER 3.2

SAVING THE ENDANGERED NAMAQUA AFRIKANER SHEEP BREED IN SOUTH AFRICA THROUGH CONSERVATION AND UTILIZATION

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INTRODUCTION

South Africa is home to a considerable number of indigenous livestock breeds that are well adapted to the challenging local environmental conditions. These include cattle (e.g. Nguni), sheep (e.g. Namaqua Afrikaner and Damara), and goat (e.g. Zulu) breeds that can withstand extreme temperature, low rainfall, seasonal variation in grassland quality and endo- and ectoparasites. They fulfill an important role in subsistence farming and smallholder agriculture with regard to cultural practices, financial stability and food security (Anderson, 2003).

Indigenous breeds are a valuable genetic resource, especially in their contribution to the overall genetic diversity and stability of rural agriculture. These breeds are particularly important for their desirable traits that allow for adaptability to harsh and arid environments, which may become more widespread in future with global climate change. Unfortunately, these breeds are often disregarded because of their perceived lower production potential compared with international commercial breeds. Uncontrolled crossbreeding, changing land-use patterns and the loss of indigenous knowledge all contribute to the potential extinction of such breeds (Hanotte and Jianlin, 2005). In order to ensure the survival of indigenous breeds it is imperative to describe their observable traits (i.e. phenotypically) and production parameters. This “phenotypic characterization” is a key initial step in the conservation of indigenous breeds, based on identification and documentation of a representative sample of individual animals - see FAO (2012) for more information. A second critical aspect of breed characterization is “genetic characterization”, the study of the molecular genetic diversity based on analysis of DNA samples (FAO, 2011).

The South African Namaqua Afrikaner sheep breed was on the brink of extinction and a project was therefore launched to save the breed through a combination of conservation and sustainable utilization. Characterization was a key part of this project, and provided critical information for the design of technically sound interventions.

PROBLEM STATEMENT

The Namaqua Afrikaner is one of the most robust sheep breeds in South Africa. It is well known for its longevity, and its performance under extreme conditions compares favourably with other South African breeds such as the Afrino and Dorper (Snyman *et al.*, 1993). The original Namaqua Afrikaner sheep migrated south with the Khoikhoi people and entered South Africa between 200 and 400 AD (Cloete, 1978; Ramsey *et al.*, 2000). The Nama people of the north-west Cape and southern Namibia raised these sheep as a key component of their culture and livelihoods and the Namaqua Afrikaner became well adapted to the harsh, dry climate of the area.



From left to right
 Brown headed Namaqua Afrikaner ewe
 @Gretha Snyman
 Brown headed Namaqua Afrikaner ram
 @Gretha Snyman

Despite its positive characteristics, this indigenous fat-tailed sheep is currently considered endangered according to FAO's risk classification system for livestock breeds. In 1995, there were approximately 2 000 Namaqua Afrikaner sheep left in the country and more recent surveys suggest that the population has declined in number since then (<http://dad.fao.org>). Moreover, poorly planned programmes aimed at improving production and uncontrolled cross-breeding have contributed to genetic dilution and the loss of genetic variation within the breed.

The main role of the Namaqua Afrikaner is to provide meat, skins, fat and hides to the rural communities in the semi-arid Northern Cape Province of South Africa. The government has recognized the breed's importance, and over the years has undertaken various support programmes. Two Namaqua Afrikaner flocks of approximately 110 ewes each are kept at two experimental stations in the Northern Cape Province. The purpose of these nucleus flocks is to provide replacement rams of good genetic quality to the keepers of Namaqua Afrikaner sheep.

During the late 1990s, the National Department of Agriculture: Directorate Grootfontein Agricultural Development Institute (GADI), tried to identify people owning Namaqua Afrikaners to establish a breeders' association. However, the majority of the Namaqua Afrikaner keepers have traditionally been smallholders, and most people only keep a few purebred animals. Only

three commercially viable farmers with flocks of approximately 100 ewes each were identified. Given the importance of local breeds to the livelihoods of small-scale livestock keepers, GADI implemented a programme (GADI-Biobank) involving the conservation and improvement of South African sheep breeds. Stakeholder workshops to discuss the issue of conservation of the endangered Namaqua Afrikaner breed were held provincially and nationally in 2006 and 2007. The two departmental Namaqua Afrikaner flocks, as well as one of the privately owned flocks, were part of this initiative. These three nucleus flocks comprised most of the remaining purebred Namaqua Afrikaner sheep in the country, and formed the base population for a breed conservation and development programme.

The project was designed to address both the needs for phenotypic and genetic characterization and to ensure the utilization and conservation of this breed. First, the breed was described and its production potential was measured so as to motivate their keepers with reference to the inherent value of the animals. A genetic study based on the DNA of the base population was also performed to establish the genetic diversity of the remaining purebred animals. This information is essential to preventing inbreeding in the long term. A cryopreservation bank for the breed was also set up.

APPROACH USED

A project for the conservation and utilization of the breed was initiated in 2009-2011, during which phenotypic and genetic characterization was performed. The study was funded by GADI and the research was carried out in collaboration with the Department of Animal and Wildlife Sciences, University of Pretoria. The biotechnology used in the project consisted of DNA markers. Microsatellite markers were used due to their usefulness in providing molecular information for small stock management (Boettcher *et al.*, 2010).

The genetic and phenotypic characterization was carried out using samples from animals of the two Namaqua Afrikaner flocks maintained at the Carnarvon (30°57' S, 22°8' E) and Karakul (28°24' S, 21°16' E) Experimental Stations, and of the third Namaqua Afrikaner flock kept by a commercial farmer at Welgeluk (WGK; 31°5' S, 21°8' E) in the Carnarvon district. These flocks are contributors to the GADI-Biobank. Blood samples were collected from 48 animals (10 rams and 38 ewes) from each flock, for a total of 144.

DNA was extracted at GADI from whole blood, and quantification was performed using a Nanodrop ND-1000 spectrophotometer at the University of Pretoria's Department of Genetics. DNA samples were amplified with 20 microsatellite markers recommended by the FAO and

the International Society of Animal Genetics (FAO, 2011). Markers were selected on the basis of amplification success, the expected allelic size range and their previous inclusion in sheep characterization studies. PCR and genotyping were performed at the University of Pretoria Department of Animal and Wildlife Sciences, Animal Breeding and Genetics laboratory. Statistical programs including MS Toolkit (Park, 2001), Arlequin (Excoffier *et al.*, 2005), Genepop (Raymond and Rousset, 1995) and Structure (Pritchard *et al.*, 2000), were used to evaluate genetic diversity, population structure and inbreeding.

Standard management practices were followed for all flocks, and the production and morphometric data collected in the Carnarvon flock were analysed to describe the phenotypic characteristics. This information is vital for promoting the breed to small-scale keepers. Namaqua Afrikaner lambs tended to be light at birth (4.2 kg), but grew quickly, so that weaning weights (26.2 kg) compared favourably with other indigenous breeds, which suggests that the breed is well adapted and capable of producing lambs that are suitable for commercial production systems.

Biotechnology has also been used for the *ex situ* conservation of the breed. A cryoconservation programme for the endangered Namaqua Afrikaner sheep breed involving collection and freezing of 307 embryos was started in 2008. Cryoconservation of semen was also undertaken, but with limited success.

IMPACT OF BIOTECHNOLOGY

The molecular characterization of the various Namaqua Afrikaner flocks provided information that has been used to evaluate and maintain genetic diversity and to control inbreeding in the nucleus flocks. This has direct value for small-scale livestock keepers, ensuring that they receive good-quality sires from the nucleus flocks through the ram distribution programmes. The genetic diversity was estimated with reference to the observed heterozygosity (a measure of the total genetic variation in the population), which yielded an average value of 50 percent. In these flocks the heterozygosity was expected to be low due to the small population size and to the flocks being kept as closed populations. The genetic diversity can be increased by using new genetic material from other sources, provided that the rams used are pure Namaqua Afrikaner and unrelated to the current flocks. The population structure analyses identified the three flocks as distinct genetic groups. This implies that there is merit in keeping the three nucleus flocks separately, and that breeding stock can be exchanged among the flocks without compromising diversity.

The inbreeding (estimated using markers by computing the F_{IS} statistic) across the population was low ($F_{IS} = 0.019$). This result indicates that the cyclic mating system applied was successful

in limiting the mating of related animals in the relatively small flocks. Current plans are to evaluate the inbreeding of these flocks every five years to determine at an early stage any unfavourable trends, so that appropriate steps can be taken to prevent further increases in inbreeding. It is also proposed that the current system of selection of replacement ewes and sires – where only animals that have physical deformities and do not conform to the general breed appearance are culled – should be continued. This will ensure that healthy breeding stock, and especially rams with high-quality genetics, can be distributed to smallholders and keepers, maintaining the levels of diversity in the national population.

As noted earlier, 307 embryos have been obtained from Namaqua Afrikaner ewes and are cryoconserved in the GADI-Biobank. These ewes were exclusively from the Carnarvon Experimental Station. Keeping in mind the genetic distance between the flocks, plans are under way to ensure that embryos from all flocks can be cryopreserved as part of future conservation activities.

PROGRESS TO DATE

Since the start of this project, another two Namaqua Afrikaner flocks have been included in the conservation programme. These flocks are also available for the cryopreservation and donation of blood for DNA storage at the GADI-Biobank. One of the flocks is kept near Calvinia in the Northern Cape Province, and the other at Barkly-East in the Eastern Cape Province. The Barkly-East flock has genetic ties with the Carnarvon flock, as the owner purchased some animals from that flock between 1994 and 1996, and again in 2010. The same set of microsatellites used in the original analysis should be used to genetically characterize animals from these two new flocks, in order to determine their genetic diversity and distance from the three flocks already characterized. If the animals in these flocks are sufficiently diverse from the flocks included in this study, the possibility of introducing rams from these flocks into the Carnarvon or Karakul flocks will also be considered in future.

The phenotypic production and reproduction performance of the Namaqua Afrikaner sheep indicated that their growth rate and reproductive performance compared well with other commercial sheep breeds. By combining the conservation effort with a commercial application, the future existence of the breed can be assured.

As an additional part of the conservation effort, a programme was implemented in 2011 where surplus young ewes and rams from the two experimental flocks are made available to farmers interested in conserving this breed, as well as to small-scale farmers in the Northern Cape Province. Sheep provided to the farmers have been selected on their production, and records

are kept with regard to their original flock and distribution. Six farmers already received a total of eight rams and 50 ewes from the Carnarvon flock. Three of these are small-scale farmers, one is a commercial farmer and the other two are conservation agencies. The success of the distribution is monitored by tracking the utilization by the small breeders and obtaining feedback on the perceived value of the programme. The programme is very much at the initial stage of implementation, and there is a continuous effort to distribute more breeding stock to small keepers in the Northern Cape Province.

PROS AND CONS

Genetic biotechnology was essential in enabling the estimation of the genetic diversity of the Namaqua Afrikaner sheep. Breed and production information was collected concurrently for the accurate description and the informed and efficient conservation of the breed. Reproductive biotechnology holds a long-term benefit for the conservation of the endangered Namaqua Afrikaner breed. The GADI-Biobank has the capacity to store up to 1 000 embryos, and efforts are under way to obtain the funding necessary to support the collection of the additional material. The embryo donors will be selected based on pedigree and phenotypic data recorded within the experimental flocks.

Conserving and developing a breed such as the Namaqua Afrikaner in a country with a dual agricultural sector serving both the commercial and smallholder agriculture sectors is not an easy task. It is essential to use modern biotechnology first to gain an understanding of the breed with regard to its genetic structure, and secondly to generate commercial interest for funding. The identification of suitable farmers interested in participating in the utilization programme poses certain challenges due to different levels of education within the communities, limited infrastructure and a shortage of agricultural extension services. There is, however, a concerted public and private effort to ensure the success of this programme.

In conclusion, biotechnology has laid the foundation for developing proper strategies for the long-term genetic management of this endangered breed. The experimental populations are kept to prevent extinction and to monitor genetic variability. At the same time, the recording of phenotypic production is essential for utilization to ensure that breeding animals distributed to small farmers add to their livelihoods and food security.

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Patagonian
Angora goat
breeder with
his flock
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CHAPTER 3.3

APPLICATION OF ARTIFICIAL INSEMINATION WITH FROZEN SEMEN IN AN ANGORA GOAT BREEDING PROJECT IN NORTHERN PATAGONIA, ARGENTINA

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INTRODUCTION

This case study describes an innovative approach for improving the quality and quantity of mohair production by smallholder breeders of Angora goats in northern Patagonia (Argentina), through the provision of training, the organization of livestock keepers and the application of reproductive biotechnologies.

Northern Patagonia is the home to approximately 550 000 Angora goats. The goats are typically raised in an extensive production system with low inputs. Nearly 6 000 goat breeders base their subsistence economy on the mohair produced by these animals. Mohair production in Argentina is mainly concentrated in the Neuquén, Río Negro and Chubut Provinces. Smallholder Angora goat farming is characterized by limited resources, with restricted access to appropriate technology, credit and infrastructure. Farmers have little or no access to the benefits of formal organization through cooperatives or breeders' associations. This situation results in feeble production rates in terms of mohair quantity and quality, and a weak negotiating capacity in marketing.

The Secretaría Nacional de Agricultura, Ganadería y Alimentación created the “Mohair Programme” for the Angora goat system in 1998, the main objective of which was to increase the standard of living of the small farmers that rear Angora goats by providing technical advice and commercial and financial assistance. The following were the specific objectives:

- Improving the quality and quantity of mohair;
- Strengthening the organizations of Angora smallholders;
- Establishing a transparent and advantageous market for mohair products.

The development of a “Breeding Project for Angora Goats in northern Patagonia” as part of the Mohair Programme aimed to increase qualitative and quantitative fibre production. Although Argentina is the world's fourth largest producer of mohair, the fibre yielded per animal is low. In addition to low individual production (ca. 1 kg/animal), raw mohair in the region is discounted commercially as a result of its large medullated fibre contamination (10 percent), resulting in lower prices for farmers.

The implementation of the animal breeding project for genetic improvement required the intensive use of superior males, which were evaluated and selected with reference to key production traits. The availability of genetic material was conditioned by numerous factors such as breed, the number of available males and their level of libido and semen production

(Ritar *et al.*, 1992). Additionally, the scope of the breeding project was influenced by the wide geographical dispersion of flocks and by the restricted Angora breeding season caused by the photoperiodism associated with the region's high latitude. The use of artificial insemination (AI) in the breeding project significantly reduced these drawbacks and contributed to providing a technical option for smallholders to improve their mohair production through access to genetically superior material. From another and perhaps even more important perspective, AI promoted the spread of superior genes to larger goat populations.

The logistics of the Angora breeding project were established through close collaboration and consultation with goat breeders. Meetings to promote the organization of the Angora smallholders and to develop and evaluate the project goals were conducted on a regular basis.

In addition, various aspects influencing mohair quality were addressed through training and extension activities. The activities included steps for the prevention of quality loss at shearing, the conditioning and subsequent classification of the mohair; and marketing approaches and opportunities. As farmers traditionally sold small individual lots fetching low prices, the Mohair Programme promoted the large-scale collection and storage of certified quality mohair. The marketing of larger lots and the assurance of the quality provided by the Mohair Programme boosted prices (Abad *et al.*, 2002; Abad, 2007).

BREEDING PROGRAMME AND ARTIFICIAL INSEMINATION

For many years, the genetic improvement of Angora goats in Argentina was organized according to a classic genetic "pyramid" scheme, whereby most of the genetic improvement occurred within a relatively small cluster of animals at the "top" of the pyramid, and improved genetic material was then multiplied through breeding in larger, unselected populations at the "bottom". A single nucleus flock (INTA Angora goats) was the only genetic centre for the improvement of Angora breed. This nucleus herd could realistically provide no more than 30 to 40 genetically superior males for natural service per year, and even the use of AI with fresh semen could not cover high demand of smallholder goat farmers for superior genetics during the short breeding season in Patagonia (two months).

The application of two biotechnologies was therefore the best way of dealing with this challenge. First, cryopreservation of semen permitted the storage of genetic material and thus facilitated improved planning and timely insemination in outlying goat herds. Second, oestrus synchronization and laparoscopic AI (LAI) improved the efficiency and success of AI. LAI allows

for the deposition of semen in the uterine horn, permitting the reduction of the sperm dose concentration relative to cervical AI (CAI) (100 vs. 200 million spermatozoa/inseminated goat) (Gibbons *et al.*, 1997), while still yielding a significant increase in pregnancy rates (52 percent vs 69 percent respectively for CAI and LAI) (Gibbons *et al.*, 1997). This increase was similar to that reported by other workers: a 20 percent increase in pregnancy rates when applying LAI in relation to average values of CAI (Cashmere: Ritar *et al.*, 1990; dairy goats: Vallet *et al.*, 1992).

The following aspects were particularly taken into consideration for the implementation of the LAI procedure with frozen semen:

1. As the combination of semen cryopreservation and LAI reduced the number of males required for reproduction, it was possible to increase the selection pressure by selecting the top 15 bucks per year, rather than the top 30 to 40. The bucks were selected each year from the INTA Angora nucleus. Firstly, genetically superior males were identified and checked clinically and serologically to ensure that they were negative for brucellosis. Then, physical evaluation for breeding soundness was performed, with special attention being given to the reproductive organs and body development. The capacity to mate and semen characteristics (quality and viability), were also considered. Finally, the capacity of bucks to be sperm-donors (willingness to be collected with the artificial vagina) and the resistance of their semen to freezing were also evaluated. Individual variation in response to freezing processes (Watson, 1995) was a major tool for the selection of males involved in the breeding project. In general, the main causes of rejection of bucks were the incapacity to ejaculate in the artificial vagina (low libido), low semen volume and sperm concentration, and low post-thaw semen quality (Gibbons, 2003).
2. The eight top goat breeders of Neuquén and Río Negro Provinces were selected, establishing the “Multipliers’ group” of genetically superior material. These eight top Angora goat breeders had approximately 700 females, ensuring the production of a sufficient number of superior males to cover the required genetic demand while maintaining sufficient genetic variation to avoid inbreeding. In addition, selection criteria for females were adopted and applied. The breeding objectives included fleece weight, the rate of medullated fibre, fleece density and average fibre diameter.
3. As a consequence of LAI implementation in the multipliers’ group, a more rational use of the genetic material was achieved, allowing the reduction of genetic differences between the INTA Angora nucleus and the smallholder herds.
4. Specific protocols for reproductive biotechnologies including semen cryopreservation, oestrus synchronization and AI, appropriate for the Angora breed of the northern Patagonia production system, were developed.
5. Technical staff were trained to carry out the project activities, on topics including the selection of males and females, the collection and processing of semen and the application of reproductive technologies.

As a complementary activity of the breeding project, INTA continued supplying superior males to smallholders at the bottom of the genetic pyramid. These males were used for hand-mating in pens to meet the growing demand for improved genetics.



Patagonian Angora goats in the INTA nucleus flock
©Martín Abad

The following is a summary of the reproductive methodology performed in the Angora goats breeding project carried out during three years in northern Patagonia (Gibbons and Cueto, 2011):

In the mating season (April-May), fieldwork included the animal selection, oestrus synchronization and AI of approximately 700 goats per year in the eight herds of the Angora multipliers. The oestrus synchronization procedure consisted of the insertion of intravaginal sponges with 60 mg of medroxyprogesterone acetate (MAP) for 17 days followed by the intramuscular administration of 100 IU equine chorionic gonadotropin (eCG) at the time of sponge removal. The onset of oestrus was detected with the aid of adult teaser bucks (4 males per 100 females) at 24, 36, 48 and 60 hours after sponge withdrawal.

The LAI technique was then carried out at 48 hours in goats showing oestrus at 24 and 36 hours, and at 60 hours in females that showed oestrus at 48 and 60 hours after sponge withdrawal. This synchronization and insemination scheme gave the possibility of inseminating all goats of one multiplier herd in one day only, reducing the number of workdays in the insemination schedule. Animal welfare was considered by using LAI under sedation and local anaesthesia. Ethical concerns were taken into account by adhering to local animal welfare regulations and practices.

The overall pregnancy rate in the multipliers' herds during the three-year breeding project was lower (54 percent) than that obtained in the INTA experimental flock under optimal management conditions (69 percent) (Gibbons *et al.*, 1997), but the reproductive efficiency (kids born/inseminated goats) of 67 percent was deemed acceptable for the breeding project (Table 1).

Table 1. Reproductive efficiency by laparoscopic artificial insemination with frozen semen in Angora goats of northern Patagonia over three years

Goats in oestrus / Goats synchronized (%)	1 690/1 964 (86)
Pregnant goats / Inseminated goats (%)	677/1 252 (54)
Kids born / Inseminated goats (%)	842/1 252 (67)
Kids born / Pregnant goats (%)	842/677 (124)

The implementation of AI with frozen semen in the Angora breeding project in northern Patagonia allowed the intensive use of genetically superior males, producing a total of 842 offspring in three years. Female kids remained under the care of multipliers, and male kids were given to the smallholders at the bottom of the genetic pyramid.

Thus, the number of animals in natural service before the implementation of the breeding project was 503, it increased to 1 292 at the beginning of the project (hand-mated plus AI) and reached 1 930 female goats under genetic improvement in the third year of its execution (Abad, 2007).

As a consequence and with the support of the Mohair Programme, the volume of certified quality mohair increased from 4 200 kg collected from 19 smallholders in 1998, to 90 000 kg from 830 smallholders in 2006. In parallel, since 2003, a differential price for quality has been offered, promoting the growing participation of smallholders in the genetic breeding project (Abad, 2007). In 2010, a lot totalling 15 000 kg of mohair fibre of different qualities was offered in the local market and for export to South Africa. After receiving several offers to purchase the lot, the final selling price, averaged across different categories certified by fineness, length and quality, was 40 percent higher than the producers had been able to obtain by selling individual lots of unclassified mohair. The commercial gains confirm the importance of working together with organized goat breeders to obtain an improvement in the quality, quantity and price of mohair produced.

LESSONS LEARNED

The use of AI must first be evaluated with reference to its chance of implementation and its limitations in regard to the specific production system (extensive or intensive and kind of production). A good understanding of the physiology of goat reproduction is also needed to improve reproductive efficiency and establish different productive strategies. For each breed and production model, it is essential to know how changes in reproductive aspects of females (development of puberty, oestrus cyclic activity, changes in ovulation rate, etc.) and males (puberty, libido, semen quality, etc.) occur throughout the year in parallel with environmental variables.

Once the decision has been taken to address a genetic improvement programme through AI, aspects such as the nutritional and health status of females and the processes of collecting, processing, freezing and storing semen should be accounted for to avoid failures that may otherwise be wrongly attributed to animal-related factors (Corteel, 1974; Lebouef *et al.*, 2000). Moreover, protocols of oestrus synchronization and AI must be adapted to different breeds and production systems to ensure that these reproductive biotechnologies become an efficient tool for each goat breeding programme.

The experience obtained during the development of this programme showed us the great importance of the participation of the goat breeders' associations in the logistics surrounding the implementation of AI as a breeding tool.

Finally, the development of the Angora goat production system has its future in the ongoing challenge to increase investment in the acquisition of valuable genetic material (bucks, semen and/or embryos), with a focus on quantitative and qualitative characteristics that can add value to its production, considering the protection of genetic biodiversity in the farming system.

ACKNOWLEDGEMENTS

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Low-cost, well-ventilated housing that yields good cow comfort (Photo taken in Summer 2011 in the Chittagong district)
©CDVF /Mohammed Shamsuddin



CHAPTER 3.4

USE OF ARTIFICIAL INSEMINATION IN A COMMUNITY-BASED APPROACH TO DELIVER CATTLE PRODUCTION-RELATED VETERINARY SERVICES IN FOUR DAIRY-PRODUCING AREAS OF BANGLADESH

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BACKGROUND

Agriculture plays a significant role in the economy of Bangladesh. The contribution of livestock to the agriculture sector is 15 percent, which is 2.5 percent of the national GDP. About 15.4 percent of the total protein consumed comes from livestock products, which increased by 4.3 percent during the period between 1990 and 2008 (FAO, 2010). The country possesses 23.2 million cattle and 1.4 million buffaloes. About 25 percent of its 150 million people are directly dependent on livestock and another 50 percent receive indirect benefits from livestock for their livelihoods (Ali and Rahman, 2010).

Over the last two decades, Bangladesh has experienced a major shift in the purpose of rearing cattle. Farmers now rear cattle and buffaloes primarily for milk and meat production, whereas prior to the 1990s these animals served largely as draught power for crop cultivation and rural transport. Livestock farmers in Bangladesh are overwhelmingly smallholders with one to three animals, and most of them own little or no land. The animals are mostly fed on crop residues and other by-products. The production of native zebu cattle (*Bos indicus*) of Bangladesh is low compared with crossbred (*Bos indicus* x *Bos taurus*) cattle.

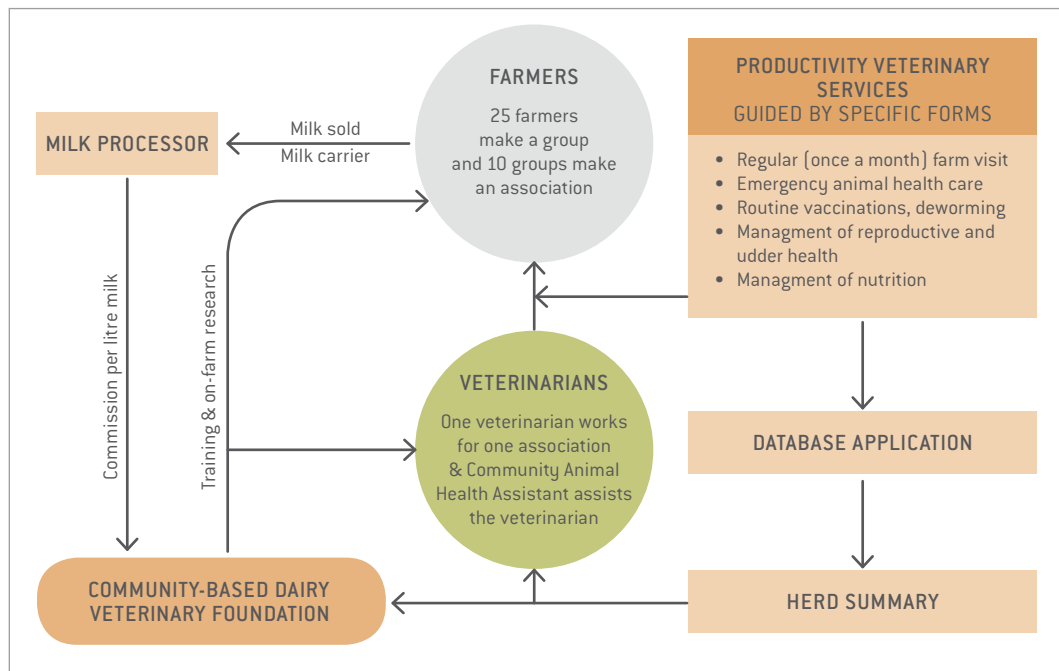
Bangladesh has been practicing artificial insemination (AI) since the late 1950s. The objective was to improve the productivity of native cattle and used semen from imported zebu, *Bos taurus* and crossbred bulls. AI activities resulted in the production of about 3 million crossbred cattle in the country. However, the impact of AI on increasing milk production remained far below the expectations of stakeholders. Bangladesh has had only a modest average growth rate (2.0 percent) in milk production over the past decade, whereas a rate of at least 6.0 percent is required to achieve a goal of making 120 ml milk available daily per capita by the year 2025, considering a population growth rate of 1.6 percent (Shamsuddin and Rahman, 2009).

High-yielding cattle, especially crossbreds involving exotic breeds, suffer more from health problems and need more inputs of feed and health care than do native, locally-adapted cattle. Artificial insemination, coupled with locally based selection programmes and herd health services, has made possible manifold increases in milk production in countries with large dairy herds with stable milk markets. The owners of large herds generate enough revenue to support the purchase of supporting services. Such services have not been consistently delivered in smallholder production systems, in part because no mechanism exists for collecting revenue to bear the expenses of the services. This problem is exacerbated when no formal channels for milk marketing are available. To address this problem, starting in around 2000, scientists at the Bangladesh Agricultural University worked with local stakeholders to establish a Community-

based Dairy Veterinary Foundation (CDVF) to deliver production-related veterinary services in four dairy-producing areas of Bangladesh (Satkhira, Sirajgonj, Chittagong and Mymensingh).

Figure 1 shows the model of the CDVF operation. Farmer associations deliver milk to chilling centres set up by the Bangladesh Rural Advancement Committee (BRAC) Dairy and Food Project, and revenue for the CDVF is generated through a negotiated commission per litre of milk delivered. The revenue is then used to pay dues for services to the CDVF. The CDVF delivers a package of on-farm activities, not only to prevent infectious and zoonotic diseases, but also to sustain production by improving animal nutrition, reproduction, udder health and welfare. This combination of activities is defined as a productivity veterinary service (PVS) (Shamsuddin *et al.*, 2010).

Figure 1. The model of delivering CDVF service in smallholder dairy farms



The PVS is not a substitute for traditional on-demand veterinary practices, rather it is a complementary addition. The PVS considers the entire herd and production system instead of focusing solely on clinically ill animals. The primary objective of PVS is to reduce the economic loss of farms due to diseases that result from sub-optimal management. The secondary objective of PVS is to reduce the economic loss from failure to attain optimum productivity with respect to milk, meat and calves.

The objectives of PVS are achieved by optimizing:

- > the health status of a herd by improving health, reproductive efficiency and production;
- > the productivity of the herd by improving management practices;
- > production process in relation to animal welfare and the ecological quality of the farm environment;
- > the quality and safety of dairy and meat products; and
- > the profitability of the livestock enterprise, either by increasing farm income or by reducing costs, or both.

In the community-based approach to smallholder farmers, the veterinarian interacts with farmers to further increase farm profits by:

- > reducing somatic cell count through mastitis prevention and control;
- > increasing dry matter intake through nutrition management and improved cow comfort;
- > improving reproductive efficiency;
- > decreasing the age at first calving through heifer management programme; and
- > advising on the effective utilization of labour and resources.

All these activities, together with guaranteed milk marketing, increase farmers' compliance with the requisites of the AI service-providers, who are now aiming to introduce genetic improvement programmes to speed up breed development.

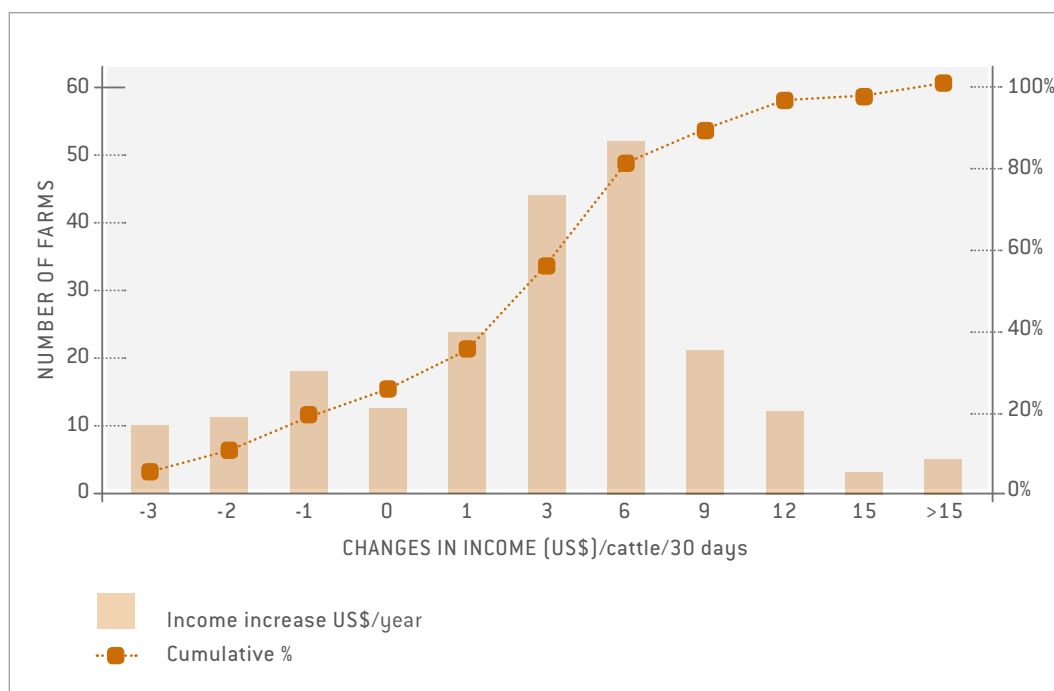
STATUS OF ARTIFICIAL INSEMINATION (AI) SERVICES IN BANGLADESH

About 4.0 million AIs were performed in cattle in 2012 in Bangladesh, of which 2.6 million were by the government through the Department of Livestock Services, 1.3 million by BRAC, an NGO, and 0.1 million by the Bangladesh Milk Producers Cooperative Union Limited, with its brand name "Milk Vita". Currently AI services can cover 40 percent of breedable cows in Bangladesh (Shamsuddin, 2011a). In a study that included major areas with dairying as an important economic activity and used the Artificial Insemination Database Application for Asia (AIDA Asia) information system to record AI data (Garcia, 2002), the conception rate in smallholder cows was 51 percent (Siddiqui *et al.*, 2013). A similar conception rate was reported earlier by Shamsuddin *et al.* (2001) who used radioimmunoassay of milk progesterone for the evaluation of the cyclicity. However, the quality of the service is not consistently high across the country. Conception rates vary widely according to the skill of the AI technician (Siddiqui *et al.*, 2013). Proper bull monitoring and culling would also

improve fertility (Siddiqui *et al.*, 2008), as would improvement and standardization of semen collection, processing and storage procedures (Sugulle *et al.*, 2006).

However, there are some obstacles at the cow and herd level. The nutritional condition of cows and buffaloes significantly affects the interval from calving to first service and is often substandard (Shamsuddin *et al.*, 2001; Banu *et al.*, 2012; Siddiqui *et al.*, 2013). Poor heat detection is also an important limiting factor. In another study using progesterone radioimmunoassay, Shamsuddin *et al.* (2006) observed that oestrus was accurately detected in only 30 percent of cases. Another 30 percent of cows were detected as in oestrus when they were not (false positive) and 40 percent cows remained undetected when they were in oestrus (false negative). Poor oestrus detection has also been reported in buffaloes, where the cyclicity was evaluated by determining progesterone in milk by using enzyme-linked immunosorbent assay (ELISA) (Banu *et al.*, 2012).

A PVS can be used to propose and deliver interventions to increase the proportions of postpartum cows cycling and bred (Shamsuddin *et al.*, 2010; Kamal *et al.*, 2012). From 47 to 86 percent of anoestrous heifers and cows resumed their oestrous cycles, evident by behavioural signs and per-rectal palpation of the genital tract, when treated with hormones, vitamins A, D₃ and E or nutritional supplements (Shamsuddin *et al.*, 2010). Ultrasound of genital tracts revealed that 53 percent of apparently anoestrous (silent oestrus) cows were in fact cycling (Kamal *et al.*, 2012). Farmers were subsequently able to breed 68 percent of the silent oestrous cows after treatment with hormone or nutrition supplementation or by increasing the frequency of visual heat detection and 70 percent of inseminated cows conceived (Kamal *et al.*, 2012). Thus, the PVS helped to increase the number of cows inseminated per year, which in turn led to an increased number of calves born, more cows in milk and higher income for the farmers. In a comparison to the period without the service, the PVS resulted in a per-day increase of 1.5 litres of milk per buffalo, 1.0 litres of milk per crossbred cow and 0.75 litres of milk per indigenous cow (Shamsuddin, 2011b). About 80 percent of farms that received the service in the Satkhira district saw an increase in their income, ranging from US\$1 to \$19.4 per cow per month (Figure 2). Moreover, with accumulated increased income, farmers were able to increase the size of their herds by purchasing new cows.

Figure 2. Effects of productivity veterinary services on farmers' income in Satkhira (n=213)

COMMUNITY-BASED APPROACH TO DELIVER PRODUCTIVITY VETERINARY SERVICES IN SMALLHOLDER DAIRY FARMS

Unfortunately, operating a PVS involves fixed costs that usually cannot be sustained by a single or small group of individual smallholders. However, although individual smallholder farmers cannot pay the cost of PVS, members of a community of 250-300 farmers that collectively produces ~2000 litres milk per day can put aside a small portion of their individual income from milk to enable them to pay together the cost of services. This concept has been proven in Bangladesh by organizing smallholder farmers and establishing the CDVF. Organization helped the farmers not only to generate a commission to support the PVS, but also to increase their bargaining power to get a better price for the milk. Furthermore, the PVS both increased smallholder farmers' income and instilled a sense of confidence in the farm community, which in turn led to a very rapid increase in the number of farmers participating and in the amount of milk produced by the community (Figures 3 and 4).

Figure 3. Milk production in Satkhira district from April 2008 to August 2010

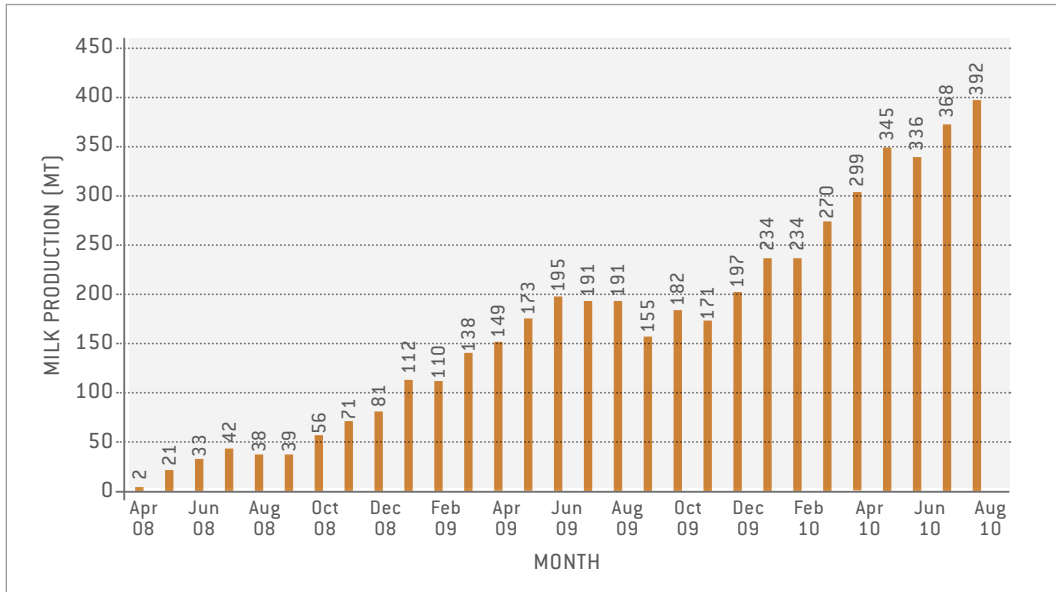
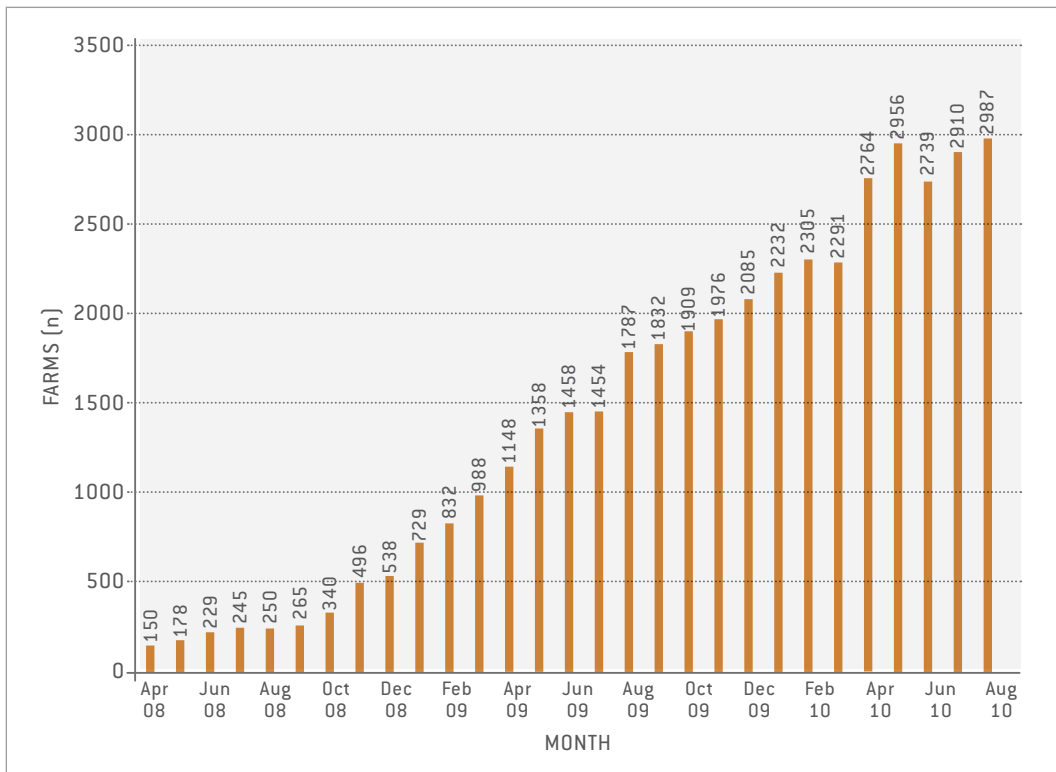


Figure 4. Number of farmers receiving productivity veterinary services in Satkhira district from April 2008 to August 2010



SELF-SUSTAINABILITY OF CDVF

A formal agreement between farmers' associations, veterinary service providers and milk processors is critical to the regular procurement of good quality milk, AI services and PVS delivery. Accordingly, CDVF has made an agreement with a milk processor in Bangladesh to install milk collection and chilling tanks for the CDVF areas in Satkhira and Sirajgonj. Milk collectors carry milk from the community to the chilling tanks twice daily. Each week, the milk processors pay a predetermined price for milk to the farmers on the basis of fat percentage of the quality-assured milk. In addition, the processor pays a commission per litre of milk to the CDVF for providing PVS to the community. The commission received from delivering 2 000 litres milk daily (from approximately 250 farms) to a chilling centre is enough to support the salaries of 20 milk collectors (US\$62.50-75 per collector monthly), one veterinarian (US\$312.50 monthly) and one field assistant (US\$100 monthly), and cover the rent and maintenance of an office (US\$75 monthly) in the community (unpublished data). In addition, CDVF provides vaccines and drugs for routine deworming for all farms. The CDVF activities led to increased income from dairying for farmers by increasing production and assuring a fair milk price. This agreement also benefits milk processors who are able to procure high-quality, natural milk, as they can test the fat and solids not fat (SNF) and check for adulteration at the chilling centre. The employment (milk collectors) generated by the CDVF is very important in a country like Bangladesh where rural unemployment is a big problem.

PRESENT STATUS OF THE CDVF

The success of the CDVF can be measured by its popularity among farmers. Between 2008 and 2012, the number of farms that received the PVS services in Satkhira increased from 150 to 2 935. In Sirajgonj, the number of participating farmers went from 170 in 2009 to 400 in 2012. Monthly milk production is currently 392 tons in Satkhira and 36 tons in Sirajgonj versus 2 and 12 tons respectively before the CDVF was established. In Sirajgonj, CDVF members had a greater ($P < 0.05$) economic return than non-member neighbours (Shamsuddin *et al.*, 2010). In Chittagong, the programme started in 2002 with 35 farmers and now involves 260. The monthly milk production increased from 75 tons to 360 tons in CDVF operation area in the Chittagong district.

THE WAY FORWARD

Artificial insemination will yield greater benefits to farmers if bulls are selected on the basis of the performance of their daughters in the relevant local production system. The herds in the farming communities of Bangladesh include a large number of locally-adapted crossbred cattle that have a large proportion of genetics imported from temperate developed countries. Many locally-adapted crossbred and indigenous animals show outstanding productivity and adaptability compared with the average population in the tropics. These cattle could revolutionize the dairy cattle breeding in developing countries because of their combination of adaptability and productivity. Unfortunately, because of the lack of performance recording programmes, animals such as these are not utilized in the county's breeding programme. The next step to advance the productivity and incomes of local farmers is to combine biotechnologies with telecommunication and information technologies. These latter technologies have created a nearly unlimited opportunity to not only collect and store information on the productivity and health of livestock and on the production system, but also to make it available to peers. Cell phones are now commonplace among farmers in Bangladesh. Mobile phone text messaging has been used to successfully accomplish many important surveys with national issues and could be used to collect and share production data. If customized to fit the purpose, these technologies can surely help to overcome the challenges of recording, retrieval, analysis and preparing reports on reproduction and breeding data in the smallholder dairy farms.

Other emerging technologies may also contribute to improve dairy productivity in developing countries. Genomic technologies can greatly improve the accuracy of genetic evaluations, assuming data is available for analysis. An important future research question will be to know how the genomes of these adapted crossbred animals have allowed them to maintain their adaptability to the hot and humid climate and at the same time incorporate effects of increased productivity, which had been imported from temperate climate.

Of course, the production system is an important issue, which is likely to differ between farms and between agro-ecological zones in the same country. Global positioning systems (GPS) and geographic information systems (GIS) can be utilized for the identification and niche mapping of the farms with outstanding breeding stocks to help account for precise effects of the environment. Radio frequency identification (RFID) and other electronic technology can be used to help identify animals. Robust reproductive biotechnologies such as ovulation synchronization guided by ultrasound of the genital tract and/or hormone assay and timed insemination will significantly increase the number of cows bred. Simultaneously, improved feeding will drive more cows to cycle for breeding with a higher conception rate. This holistic approach will bring a new era of implementing reproductive biotechnologies in the smallholder farms and recording quality data for selecting outstanding stocks for further developing AI services in future breeding.



Well grown heifers; the heifer on the left is two months pregnant at 15 months of age
©CDVF/Mohammed Shamsuddin

CONCLUSIONS

The coverage of AI in Bangladesh has increased over the last decade from 30 to 40 percent; however, the national average for milk production has remained far below expectations. Smallholder farmers obtain greater benefits from AI services if they are coupled with PVS and milk-marketing opportunities. Organization of smallholders into private farmers' associations has proved itself a driving force for the development of dairying in Bangladesh by increasing bargaining power and skills in the management of farm economics. In the future, a holistic approach combining AI and PVS with information technologies for performance recording of a critical number of animals as the future breeding stock, molecular technologies for genetic and genomic characterization of economically important traits, the application of reproductive biotechnologies such as ovulation synchronization guided by ultrasound, hormone assays and improvement of nutrition can provide new opportunities for the fast growing dairy industry in Bangladesh to provide milk to feed the people.

ACKNOWLEDGEMENTS

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Harvesting of
ferrirrigated maize
in August 2012
©EMBRAPA/Rubia Rech

CHAPTER 3.5

INTENSIVE AND INTEGRATED FARM SYSTEMS USING FERMENTATION OF SWINE EFFLUENT IN BRAZIL

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INTRODUCTION

Among the major challenges of the near-future agriculture is achieving the ability to simultaneously provide enough food and ecosystem services to urban societies, while providing “equivalent urban opportunities and benefits” to rural smallholders, who represent more than 50 percent of the global food production (Herrero *et al.*, 2010; Lee *et al.*, 2012). Meeting this challenge would considerably reduce rural exodus to the cities and, at the same time, achieve a more adequate point of equilibrium between food security and the provision of ecosystem services on Earth (e.g. Uriarte *et al.*, 2012). Biotechnologies have a complementary role to play in achieving these goals.

Large-scale, intensive farming systems usually comprise monocultures designed to provide a limited range of product outcomes. In the case of Brazil, most monocultures are focused on the production of sugarcane for sugar and/or ethanol, eucalyptus for wood and paper industries, and soya and grains for intensive production of livestock (e.g. for milk, beef, pork and chicken) and modern industrialized human foodstuffs. Although large-scale farming has been highly profitable and has contributed greatly to the Brazilian export market, this production model has numerous drawbacks, including: the concentration of land, financial wealth and other resources; increased rural exodus; and loss of ecosystem services such as water production and purification, carbon sequestration in soils and forestry and several other biogeochemical processes involving local and regional biodiversity.

Therefore, over the last two decades, Brazil has been redesigning its rural landscape by promoting the redistribution of land to smallholders. The consolidation of democracy and wide spread of syndicalism have been the catalysts for the resettlement of many families. However, the ambiguity of the syndicalism thesis and the lack of planning in government programmes have rendered the land redistribution activities largely ineffective, primarily because:

- most of the new rural settlers were already so “urbanized” that they had lost their indigenous knowledge and agricultural skills;
- the settlements were installed in areas with inappropriate or unproductive soil profiles, and, particularly;
- the services to provide technical and financial assistance to the newly settled families were grossly inadequate.

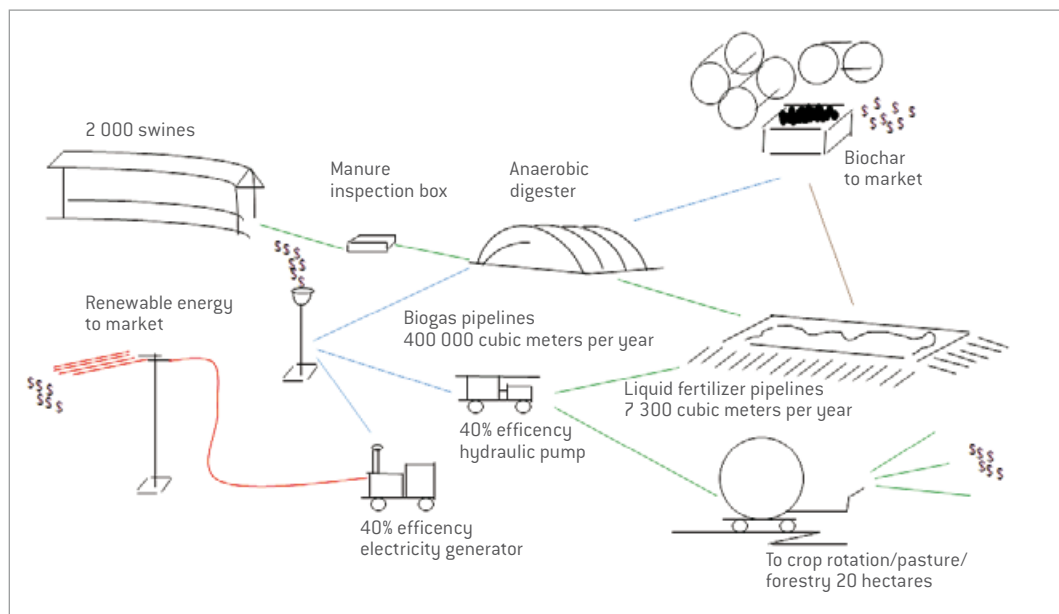
To address this problem, the Brazilian Agricultural Research Corporation (EMBRAPA) undertook a study with the Ministry of Science, Technology and Innovation (MCTI) and the Ministry of Agriculture, Livestock and Food Supply (MAPA) to identify critical factors for the successful

resettlement of smallholders. The first factor noted was the need to recognize or envisage local and regional strengths and weaknesses regarding rural productivity. This can be best achieved by inspecting local and regional natural resources and noting the presence or absence of established markets and industries and assessing the available knowledge and capacity for innovation.

THE PROJECT

The project *Intensive and Integrated Farm Systems for Smallholders* in São Gabriel do Oeste, Mato Grosso do Sul, Brazil (Campanário Settlement - 19°16'46.90"S, 54°36'2.35"W), is a good example of incorporating biotechnologies into a successful programme of re-establishing families in rural settlements, while maintaining their dignity and ensuring adequate power of consumption. The Campanário Settlement comprises 142 families on 2 850 hectares (i.e. 20 hectares per family). The core scope of the project is the maintenance of smallholder families in the food production system by having access and opportunities as similar as possible to those of families living in nearby cities or metropolises.

Figure 1. Illustration of the Intensive and Integrated Farm System driven by swine effluents tackled with innovative technologies developed in São Gabriel do Oeste, Mato Grosso do Sul, Brazil



Source: illustration by Carlos Shimata, improved by Ivan Bergier



from left to right

Biodigester effluent as a source of fertilizer and biogas-driven machinery involved in fertirrigation of integrated cropfields

©EMBRAPA /Ivan Bergier

Maize and eucalyptus tree lines fertirrigated with biodigested swine manure in April 2012

©EMBRAPA /Rubia Rech

The project is a collaborative effort involving EMBRAPA, a local cooperative called the Cooperativa Agropecuária São Gabriel do Oeste Ltda (COOASGO) and a local company (Retificadora Centro Sul) and is built on the idea that it is possible to use biotechnologies to profitably transform potential swine industry pollution into profitable and useful products while simultaneously promoting favourable balances of energy, water and nutrients with social inclusion. The excess organic material and energy from swine production is converted into new products that can be commercialized by the cooperative at favourable prices and with the sharing of profits, thus substantially increasing the revenue of the settlement dwellers.

The initiative was started in 2008 by the City Hall of São Gabriel do Oeste, underlining the importance of the local government in the success of the Campanário Settlement. At that time, COOASGO, in partnership with the city government, EMBRAPA, MCTI and MAPA started a programme of social inclusion for 13 selected smallholder farmers by enabling the construction of thousand-head swine production facilities with a shared fertirrigation system (where fertilizers are applied with irrigation water). Two contiguous swine farms were chosen to implement a pilot-farm unit with the installation of a laboratory (gas chromatography, computing and water quality monitoring), in operation since May 2012, and a facility for demonstration of power and solid fertilizer production, in operation since April 2012. The

selection of farms was based on the perceived capability of each family to follow the guidelines established by the cooperative to improve productivity, reduce the use of non-renewable resources and, consequently, mitigate the pollution of air, water and soil. A private company (Brascarbon) interested in certified emission reduction under the Clean Development Mechanism of the Kyoto Protocol also collaborated by constructing biodigesters and agreeing to transfer ownership of the digester to the settlers after seven years of operation. In addition, the digester effluent and a portion of the biogas generated were allowed to be used by the smallholders under the previously described *Intensive and Integrated Farm Systems for Smallholders* project, which is funded by the MCTI and the National Research Networks in Agrobiodiversity and Agricultural Sustainability programme (CNPq/REPENSA).

The controlled use of the anaerobic biodigestion or fermentation can be considered as one of the most important biotechnologies created by mankind. For example, it serves as basis for the production of numerous products for human consumption, silages and by-product feeds for livestock production and ethanol as a biofuel. In the case of effluents produced by concentrated populations of humans (cities) or animals (livestock), biodigestion has become a common technique for secure disposal and water treatment.

The commercial pig industry demands precise control of the materials and components of the swine diet. COOASGO has the advantage of producing its own feed with selected materials designed to optimize feed conversion efficiency. In the typical Brazilian production system, pigs are marketed after 120 days of confinement, meaning there are three cycles of swine production per year. The fermentation of swine effluent has two main outputs: 1) biogas for power generation and, 2) biofertilizer, which can fully replace mineral fertilizers in well-managed (i.e. kept at a high fertility level) soils of agro-ecosystems. The biogas is reasonably enriched in methane (>50 percent) that has an average heat of combustion of around 55 MJ/kg.

Considering that anaerobic biodigestion of animal waste can provide renewable energy and renewable fertilizer, its widespread integration into the swine industry and other agricultural sectors seems nearly inevitable, in particular in West Central Brazil. The anaerobic biodigestion of effluents from the swine industry is now so important that the traditional crop production areas of West Central Brazil are steadily becoming the main areas of swine production. This scenario favours the “Intensive and Integrated Farm Systems” project for the successful social inclusion of smallholder farms in Brazil. The project can be applied on both large and small farms; it is suitable for cooperatives and is extraordinarily effective at improving productivity while reducing pollution.

A local company named Retificadora Centro Sul has developed a unique engine (Rieger, 2006) to convert the biogas into either mechanical or electrical energy. The development was achieved after five years of exhaustive tests carried out on diesel engines adapted for biogas by means of special electronics and automation. The power efficiency of these engines is 40 percent, which is astonishingly superior to similar engines available worldwide. An adapted diesel power station of 100 kW can run continuously on inputs of 40 cubic metres of biogas per hour. Considering that a single adult pig has the potential to produce wastes yielding roughly 0.5 cubic metres of biogas per day (Bergier *et al.*, 2012a), a population of 2 000 animals can produce 100 kW of power, which is very attractive for the alternative energy market in Brazil. However, this component of the project still requires government regulation of the market at the state level to be properly implemented.

A fraction of the power is used for dispersing the biodigester effluent onto pasture, crop, or forest land or combinations of these in different proportions. This process is called fertirrigation, where a pump draws the effluent, whose annual production from 2 000 swine is about 7 300 cubic metres (Bergier *et al.*, 2012a), and moves it through stainless steel pipelines to a series of three or four manually regulated valves. At these valves, a pipeline reel has been connected that disperses the effluent over rectangular plots of three hectares each with pasture or corn, each of which are adjacent to eucalyptus groves. Retificadora Centro Sul also designed this technology, which includes a special pump driven by biogas (Rieger, 2006), so the entire processes of power generation and fertirrigation are completely driven by renewable energy. Consequently, instead of simply burning the methane to decrease the impact on climate change, the project uses it in a beneficial way, thanks to the locally-developed technology. The local knowledge in São Gabriel do Oeste was crucial not only to the sustainability of the swine production enterprise, but was also integrated into the production of other foods (such as milk, beef and grains) and material (eucalyptus wood and solid fertilizer).

THE OBSERVED AND EXPECTED OUTPUTS AND OUTCOMES

For every thousand swine, the effluent produced can safely fertirrigate an area of 10 hectares (Bergier *et al.*, 2012a). The effluent has been applied to pasture, corn and soybean fields and a small eucalyptus plantation. As a result, with fertirrigation of 180 cubic metres of biofertilizer per hectare per crop cycle, the increase in yield of the pasture land has allowed the community to double its milk production, while also increasing the productivity of corn and soya. The fertirrigated eucalyptus in this study was planted in 2011/2012. With this application rate, the

wood produced is expected to be available for the market in four years, substantially earlier than the standard seven years using conventional forestry technology.

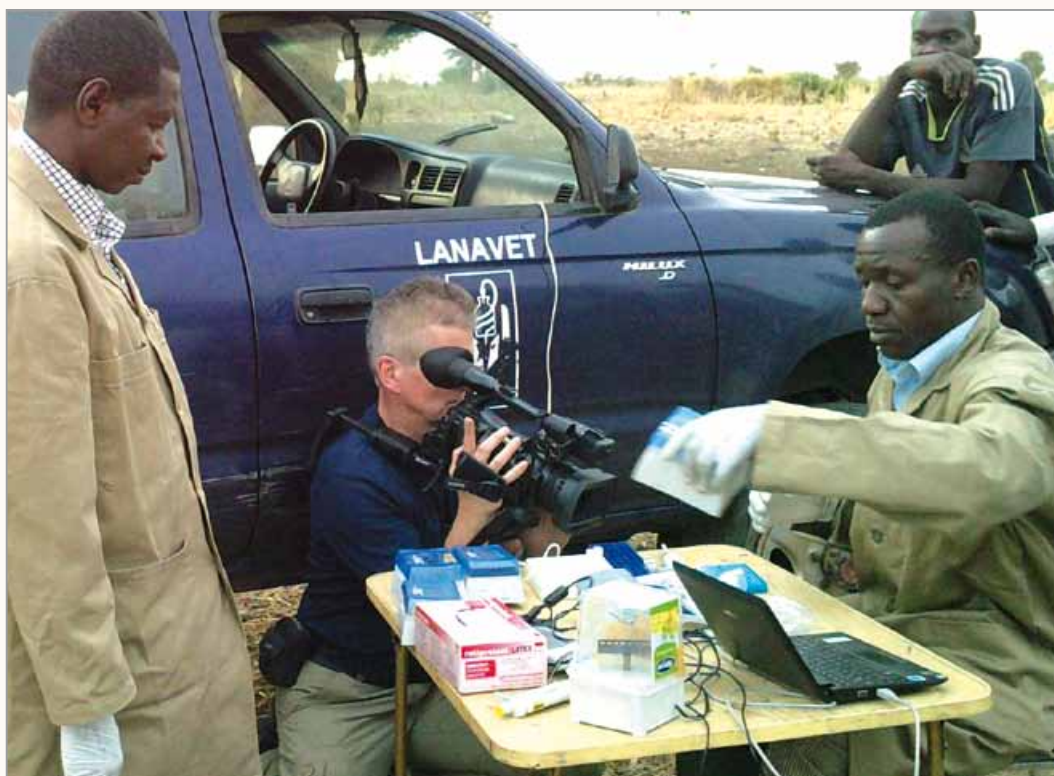
The biogas and the swine effluent are also key inputs in the process of creating a novel solid fertilizer. This fertilizer is produced by pyrolysis of the nutrient-dense (especially N, P, K, Ca, Mg and other micronutrients) digested swine solids, yielding a material with biochar-like properties. The biochar is produced under special conditions that impart to it the properties of slow nutrient release and high humidity retention, allowing it to improve soil quality and carbon content, thus improving the productivity and the sustainability of the mixed livestock and cropping system. The machinery and process of production of this special biochar were developed by EMBRAPA, Retificadora Centro Sul and COOASGO and are currently undergoing the patenting process (Bergier *et al.*, 2012b, 2012c). The machine for solid fertilizer production in the pilot-farm yields about 800 kilograms of biochar per month (unpublished data). Laboratory-scale biochar production from digested swine manure is discussed in Bergier *et al.* (2013).

All of these products (renewable power, fertirrigation of agro-ecosystems and biochar) are also contributing to the restoration of natural forestry for recovering the ecosystem services of a portion of the farmlands, in association with the National Institute of Colonization and Agrarian Reform (INCRA), as a strategy to help cope with the extreme weather events (droughts or floods and increased temperatures) expected under future climate change scenarios. The project has the vital collaboration of two government Ministries (MCTI, MAPA), universities (Federal University of Mato Grosso do Sul - engaged in monitoring groundwater; State University of Campinas – modelling by means of Life Cycle Analysis and Energy; and Federal University of Paraná – Principles of Integrated Farm Production: PISA), and institutes (National Institute for Space Research – greenhouse gas and atmospheric transport and Federal Institute of Mato Grosso do Sul – database development, sensor network development and electronics and automation knowledge transfer), with a grant from MCTI and CNPq/REPENSA .

The CNPq/REPENSA grant has been used for monitoring key processes (gaseous emissions, water and soil qualities) taking place in the pilot-farm. To inform the public about the project, a group of about 30 students from 9 to 14 years from the “Dorcelina Folador” state school in the settlement were familiarized with the project as part of a strategy to transfer the knowledge in open source hardware and software (Arduino), and, specifically, to promote the use of technology and innovation for achieving sustainability by the next generation of the Campanário Settlement.

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Laboratory analysis of collected samples (nasal swabs and blood) using LAMP PCR in the field. The LAMP device is connected to the cigarette lighter of the car (for energy) and to a laptop (for reading the results in real time)
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CHAPTER 3.6

TAKING THE LABORATORY TO THE FIELD: RAPID DIAGNOSIS OF PESTE DES PETITS RUMINANTS (PPR) IN CAMEROON

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INTRODUCTION

Peste des petits ruminants (PPR) is an acute and highly contagious viral disease of small ruminants (especially sheep and goats). The disease is economically very significant due to its very high (close to 100 percent) morbidity and mortality rates. First identified in West Africa in the 1940s, PPR is now present throughout sub-Saharan Africa, including Cameroon, and has spread to other parts of the world as well. The wide diffusion and high virulence and contagion of PPR have led to its status as a reportable transboundary animal disease (OIE, 2012; FAO, 2012).

For years, farmers have been terrified by PPR outbreaks in sub-Saharan Africa and one factor limiting its control is the lack of an early detection system complementary to the sophisticated laboratory-based diagnostic procedures. Such a tool could help stop the spread of the disease at an earlier stage, limiting its impact.

The PPR virus is an RNA virus and thus has to be reverse-transcribed before the nucleic acid can be amplified using the polymerase chain reaction (PCR). Although scientists have developed several methods of diagnosing this disease, they were all found to be technically challenging, not sufficiently robust, time-consuming, expensive and not well adapted to African reality in the field. The rapid diagnosis of a pathogen during an outbreak investigation is not only essential to ascertain the cause but, more importantly, allows fast decision-taking for the application of preventive and control measures to stop its rapid spread.

Loop-mediated isothermal amplification (LAMP) is a novel method for nucleic acid amplification in which the target nucleic acid is continuously amplified by a single enzyme at a constant temperature. In contrast, standard PCR requires a thermal cycler, a specialized machine that alternates between low and high temperatures to allow repeated turns of DNA synthesis and denaturation. Because LAMP PCR is undertaken without the need of thermal-cycling equipment, it has attracted quite some scientific interest as a practical diagnostic tool in the field, especially in developing countries (Fu *et al.*, 2011). The technique uses four primers that recognize six regions on the target nucleic acid, so that the specificity is extremely high. The LAMP PCR method is also highly efficient and enables the synthesis of large amounts of DNA in a short time (less than an hour including sample preparation). The accumulation of amplified target DNA results in increased turbidity of the reaction solution, or changes in fluorescence if specialized marker dyes are incorporated. These changes can be detected with a photometer and even measured in real time to allow for a quantitative analysis. As the readings have quantitative values, they can be sent via mobile phone to a reference laboratory

for confirmation and further study. Protocols have been developed for detection of PPR virus via LAMP PCR (Li *et al.*, 2009; Li *et al.*, 2010).

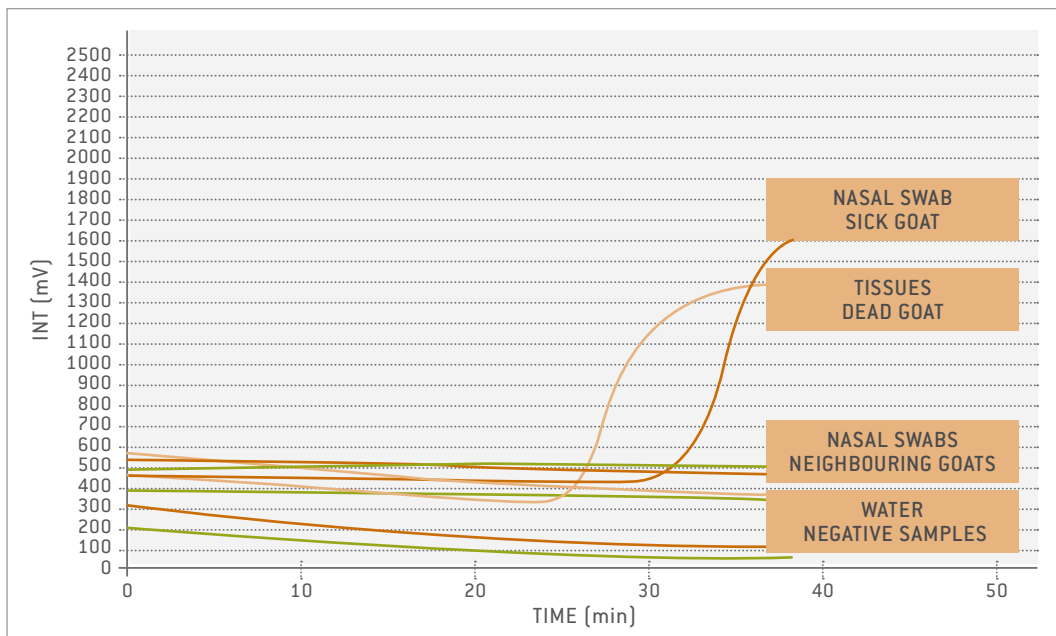
In an attempt to use this technology to assist countries under the umbrella of the International Atomic Energy Agency (IAEA) “Food for the Future” initiative (IAEA, 2012), a diagnostic procedure based on LAMP PCR was developed through a coordinated research project operated by the joint FAO/IAEA Programme for Nuclear Techniques in Food and Agriculture (AGE) in Vienna. Scientists at AGE developed a specific “Master mix” of reagents that can be transported at room temperature and used to apply LAMP PCR directly to biological samples without the need for DNA or RNA extraction. Through this project, the LAMP PCR technique was transferred to the Laboratoire National Veterinaire (LANAVET) and several other laboratories to evaluate its fitness for purpose and suitability as a field diagnostic device. In addition to these reagents, the project also distributed to participants an adapted “tube scanner” photometer, a fluorescence measurement system based on next-generation technology, for detection of the pathogen and quantification of the amplified DNA. The tube scanner used in the project is manufactured by the ESE/Qiagen company (Düsseldorf, Germany), and distributed free of charge to the countries participating in the project. These two “ingredients” provided the foundation for building simple “mobile laboratories” consisting of the tube scanner and a laptop computer for visualization of the results – both connected to a 12-volt car battery and vials of reagents and two pipettes allowing for a very rapid real-time test (results obtained within 40 minutes) with only three pipetting steps. In addition, this real-time method allows the amplification of RNA in a single closed tube. The assay system is cost-effective: the tube scanner costs about US\$6 000 and reagents about US\$2 per sample. This report presents the success story of using the mobile laboratory concept in the field to investigate two outbreaks of PPR in Cameroon.

DESCRIPTION OF THE CASES

The first case occurred in January 2012 after the death of one goat and the observation of clinical signs in several other members of a herd of sheep and goats in Vélé centre, Vélé sub-division, Mayo-Danai division in the Far North region of Cameroon. The local delegate of the Ministry of Livestock, Fisheries and Animal Industries (MINEPIA) informed the Director General of LANAVET via telephone a few minutes after the death of the first goat in the afternoon of 29 January 2012. The mobile team arrived the following morning (11.00 hours) from the LANAVET offices in Garoua, about 325 km away. After clinical examination of the herd by the LANAVET team, PPR was proposed as the disease responsible. Samples were immediately collected from sick goats and

the carcass was examined to confirm the clinical diagnosis. Samples were subjected to LAMP PCR carried out in a tube scanner (LAMP device). Positive results confirming the PPR diagnosis were obtained within 30 minutes (Figure 1). The local veterinary officer of MINEPIA was immediately alerted and rapid control measures were taken. A ring vaccination programme against PPR was organized immediately the next day for all susceptible animals in the entire village, including the neighbouring countryside. Although the diagnosis was unable to save the animals in the flock where the outbreak occurred, the quick action limited the outbreak to the original farm.

Figure 1. PPR LAMP PCR results for outbreak in Vélé: the two upward curves are for positive samples. Negative samples are linear (water negative control and swabs from goats of neighbouring farms)



The second outbreak was in Gabarey Waka (about 25 km from the first site) in the same division, along the Logon River less than two kilometres from Koumi village in Chad. It was reported to the Director General of LANAVET in the same manner as the previous one on 26 March 2012, and was also diagnosed using LAMP PCR. The subsequent implementation of a vaccination programme again contained the outbreak to the original flock and mortality in that flock was limited to 30 percent.

In addition to its simplicity, requiring neither thermal-cycling equipment nor DNA/RNA extraction, LAMP PCR is also flexible in terms of the source of biological samples. In the first outbreak, nasal

swabs from a sick goat and tissues (lung and intestines) from the dead goat were used for the analysis. In the second outbreak, nasal swabs and whole blood samples were collected from four clinically sick goats and analysed using LAMP PCR. Samples of blood for sera were also collected for further analysis.

The real-time graphics for the outbreak in Vélé is presented in Figure 1. A clear real-time curve of the amplification plots of the two positive samples was observed already after 25 minutes in Figure 1, indicating that a specific reaction for the PPR genome had happened.

DIFFERENCE MADE

The impact of the field diagnosis was very positive. It was successful not only in identifying the pathogen at the earliest stage possible, confirming the concerns of the farmer, but also and more importantly, in triggering rapid control measures (ring vaccination in communities around the infected farm, the gathering and sensitization of farmers of the risk and of the need to respect the “no-in, no-out” principle to avoid disease spread), which halted the spread of PPR at the farm level. As noted previously, all goats on the first farm contracted PPR and died, while 30 percent mortality was observed in the second flock within two weeks of the initial diagnosis. These results demonstrate the very destructive effect of that PPR viral strain. Nevertheless, with the benefit of the rapid diagnosis, damage was limited to these flocks only. Without this rapid response, thousands of sheep and goats would likely have succumbed to the disease during these outbreaks, leading to millions of CFA francs in losses. Such a fast reaction would not have been possible if the conventional procedure had been followed of taking samples to the laboratory for analysis and then waiting for results before implementing control measures.

For example, just a few years earlier, PPR destroyed almost all the goats in the nearby village of Sinasi (a locality in the Mayo-Rey division), before control measures could be implemented. Similarly, another outbreak was recorded in Guider (North region) where most of the community’s goats died from infection by the PPR virus before control action could be taken. In addition, an acute contagious bovine pleuro-pneumonia outbreak in Douang Gouvra (Mayo-Danai division) caused the death of 12 cattle (40 percent) within one week in five small household herds, with most of the rest infected by the time that control measures were finally implemented. Although one cannot be certain, it is likely that application of the LAMP PCR diagnosis procedure could have limited the damage in these cases.



Arrival of the LANAVET mobile laboratory team at the outbreak site, installation of working materials while farmers bring sick sheep and goats for sampling in order to get the diagnosis *in situ*. The senior veterinarian (Abel Wade) calls the Director General of LANAVET after clinical examination of a few animals to say that PPR is highly suspected and urgent laboratory analysis is needed
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As is nearly always the case with application of biotechnology, the success achieved was not entirely the result of the LAMP PCR technology, but depended also upon appropriate training by AGE of LANAVET staff and continual interaction among farmers, veterinarians and staff of LANAVET and MINEPIA. When farmers see how the veterinarian is conducting sample analysis in their farm, they are motivated to collaborate in control measures. As a result of this communication, the AGE was able to develop the technology to meet exactly the expectations

of veterinarians doing fieldwork and performing their tasks far away from laboratory infrastructure. In terms of lessons learned, the principle of taking the laboratory to the field is an attractive option for the surveying and control of transboundary animal diseases, especially in developing countries.

CONCLUSIONS

LAMP PCR has the potential to catalyse a revolution in the diagnostic methods used in disease surveillance and investigation in developing countries. This was the first known report of an in-the-field molecular diagnosis of a transboundary animal disease outbreak, and the response time was cut to within a few hours from the initial disease reporting. This technique should be further exploited, as its implementation will improve disease reporting and allow the rapid application of control measures if coupled with vaccination and targeted culling. Livestock farming is the main economic activity for the majority of poor households, so early disease diagnosis (taking the laboratory to the field) and the rapid control of outbreaks, as in this case study, have the potential to support poverty alleviation. Faster access to veterinary services, containment of outbreaks at the early stage and limitation of mortality rates can improve the economic situation of small livestock holders.

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The eradication of the tsetse fly *Glossina austeni* and the disease trypanosomosis from Unguja Island allowed the maintenance of upgraded cattle breeds that produced significantly more milk
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CHAPTER 3.7

APPLICATION OF THE STERILE INSECT TECHNIQUE IN ZANZIBAR TO ERADICATE TSETSE FLIES, THE VECTORS OF TRYPANOSOMOSIS

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BACKGROUND

In 36 countries in South Saharan Africa, tsetse flies (*Glossina* spp.) transmit blood parasites of the genus *Trypanosoma*, causing a deadly disease called trypanosomosis, also known as “sleeping sickness” in humans and “nagana” in livestock. An area of 8.7 million km² is infested by at least one of the more than 30 tsetse fly species and subspecies, exposing approximately 50 million African cattle to the bite of tsetse flies.

The disease is a major bottleneck to agriculture and rural development, as productive livestock needed for animal traction to plough the land and for transporting commodities to markets, as well as for the production of milk, meat and fertilizers, often succumb to the disease. Found only in Africa, tsetse flies and the disease they transmit are responsible for over US\$1 billion in direct losses to livestock every year, while the lost potential from not-realized production in agriculture and livestock systems is estimated at US\$4-5 billion per year. This is because the nagana infection – even when not fatal – weakens animals to the point that they are no longer able to produce or provide draught power for agriculture and other purposes.

The continuous treatment of livestock with trypanocides and insecticides is not sustainable in view of the high costs and increasing resistance to these drugs. Vector eradication campaigns using traps, spraying and covering livestock with “pour-on” insecticide have been effective in suppressing tsetse populations, but in areas of denser vegetation, where insecticide penetration and attraction to the traps are limited, eradication has not been achieved. Under such situations, the sterile insect technique (SIT) can be an important final component of area-wide integrated pest management (AW-IPM) campaigns, with sterile males locating and mating with the remnant wild virgin females to render areas under agricultural development completely free from the tsetse and trypanosomosis disease.

ZANZIBAR

One example of successful application of the SIT for control of tsetse and trypanosomosis is the Unguja Island of Zanzibar in the 1990s. Prior to this programme, trypanosomosis prevalence among the mostly indigenous cattle averaged around 19 percent. The rural farming communities were unable to maintain livestock as a basis for productive mixed farming, for providing sufficient and good-quality food crops and vegetables, milk and meat to their families, or for income-generation through marketing agriculture and livestock products.

During several years of preparatory activities, a monitoring network was established and the fly population was suppressed using insecticide-based control tactics such as pour-on formulations on livestock and stationary targets that attract and kill flies. This period was followed by a four-year operational AW-IPM campaign involving the systematic monitoring and weekly aerial release of sterile male flies. After the last wild female fly was trapped in September 1996, weekly sterile male releases and monitoring were continued respectively through 1997 and 1999 to ensure that the tsetse was indeed eradicated and disease transmission had stopped.

The SIT is a type of birth control for insects. It is a biotechnological tactic that integrates biological and engineering techniques to produce on an industrial scale and then release, usually by air, reproductively sterilized (usually via irradiation) insects of the target pest. Virgin female individuals of the pest population that are mated and inseminated by released sterile male insects do not produce any progeny. If the repeated inundative releases of sterile males allow them to out-compete wild males for mating, the wild population declines. The SIT acts in an inverse density dependent way. Sterile males become increasingly effective with the declining pest population in finding and mating with the remaining wild females. In situations where populations are isolated and systematic releases over the whole target pest population are sustained long enough, the population can eventually disappear. Other modern biotechnological tools such as molecular population genetics are used to study the degree of gene flow between various pest insect populations, which provides indicators regarding their relationship and potential isolation. This information on particular pest populations leads to better feasibility assessment and planning of AW-IPM campaigns with an SIT component.

To meet the demand for sterile insects, a mass-rearing facility was established at the Tsetse and Trypanosomiasis Research Institute in Tanga, Tanzania, and a colony of more than one million tsetse females was maintained during the operational phase. Because tsetse flies feed only on blood, a system to collect blood from slaughtered animals was established, and a process to decontaminate the blood from micro-organisms developed. The decontamination of blood was achieved through irradiation, using the same gamma chamber used to sterilize the males, but at a different dosage. Starting in 1994, weekly aerial release of sterile males took place over Unguja Island, following established flight lines. At the peak of the eradication campaign, 110 000 sterile males were released each week over the entire island, which drove the population to extinction. An intensive monitoring network confirmed that the wild tsetse fly population had declined and had been finally eliminated, and that transmission of trypanosomiasis to livestock had disappeared (Vreysen *et al.*, 2000).

The project was financed by the Government of the United Republic of Tanzania, IAEA, FAO, and various donors including Belgium, Canada, China, Sweden, the United Kingdom, the United States of America, the International Fund for Agricultural Development [loan to Zanzibar] and the OPEC Fund for International Development.

For decades, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, with its Agriculture and Biotechnology Laboratories in Seibersdorf, Austria, has been developing, refining and promoting this environmentally friendly technique for controlling populations of major insect pests. Since 1970, at the request of African countries suffering from this deadly disease of humans and warm-blooded animals, the feasibility of using the SIT for tsetse species was assessed. Mass-rearing, sterilization, transporting and release procedures were developed for seven tsetse-fly species of major economic importance, including an *in vitro* artificial membrane blood-feeding system, thus avoiding the large-scale use of live animals for tsetse feeding. The Tsetse and Trypanosomiasis Research Institute in Tanga further refined these techniques for the target tsetse-fly species in Zanzibar.

Over the years, the Government of Tanzania, along with external partners, had made several attempts to eradicate populations of different tsetse-fly species in the country using traps, ground spraying and “pour-on” insecticide formulations, but the suppressed fly population always rebounded. It was only after the SIT was added to the traditional methods used in Zanzibar as part of an AW-IPM campaign, that the *Glossina austeni* population was finally eradicated from the island. A centrally organized full-time management team and structure under the Ministry of Livestock and Fisheries Development of the Federal Republic of Tanzania and the Ministry of Agriculture of Zanzibar, was established to manage the fly production in Tanga and the area-wide field activities on Unguja Island and to coordinate the implementation of the campaign with livestock owners and other stakeholders.

CHALLENGES

One of the main challenges in area-wide programmes is organizing farmers into associations and getting the commitment and support for the eradication campaign from government authorities, donors and other stakeholders. Another challenge is the mass-rearing of insects, which is a seven-days-a-week/365-days-a-year effort, requiring good technical, engineering and managerial support. One of the challenges for the Zanzibar campaign was an army ant attack one night on the tsetse colony, which delayed colony build-up and, consequently, the campaign [tsetse flies have a slow rate of reproduction].



After the removal of the tsetse fly *Glossina austeni* and the disease trypanosomosis from Unguja Island, agricultural productivity increased significantly mainly due to the availability of draught oxen
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The operational budget of the AW-IPM project with the SIT component in Zanzibar was approximately US\$3.5 million spread over four years, which included insecticide-based tsetse suppression, veterinary and entomological monitoring, sterile male production and weekly aerial release. An additional US\$2 million included substantial insectary refurbishment, operational research, and many other activities that have also benefited other projects. An earlier major eradication attempt by other institutions, which spent over US\$3 million on only applying insecticide-treated targets and pour-ons on livestock, contributed to suppressing the tsetse population on Unguja, but was unsuccessful in achieving eradication. The successful eradication therefore clearly involved a substantial investment, but costs for the entire AW-IPM campaign must be weighed against the many benefits obtained so far and expected in the future, and against the cost of permanently living with the problem and investing in continuous suppression based on insecticides.

IMPACTS

With the complete disappearance of trypanosomosis, farmers on Unguja were able to integrate livestock with cropping activities in areas where this had been impossible before. Overall, the quality of people's lives increased substantially thanks to increased livestock and crop productivity, animal use for transport and traction, etc. As summarized by Feldmann *et al.* (2005), two economic surveys conducted two and five years after the completion of tsetse eradication operations (Tambi, Maina and Mdoe, 1999; Mdoe, 2003) confirmed the following.

- The number of small farmers holding indigenous cattle increased from 31 percent in 1985 to 94 percent in 2002.
- There was high demand for improved livestock breeds (mostly crossbred), and the number of farmers holding improved cattle breeds increased from 2 percent in 1985 to 24 percent in 2002.
- From 1985 to 1999, milk production nearly tripled and the proportion of farmers selling milk from indigenous cattle increased from 11 percent in 1985 to 62 percent in 1999.
- The portion of small farmers using oxen for ploughing increased to 5 percent in 2002 but was expected to increase thereafter, significantly increasing the crop productivity of their farms.
- From 1999 to 2002, the average monthly income of farming households increased by 30 percent. The proportion of households with a monthly income of over US\$25 increased from 69 to 86 percent; and the proportion with a monthly income of over US\$50 increased from 22 to 36 percent. This can be associated with tsetse and trypanosomosis eradication since a strong correlation was observed between household income and milk yields, milk sales, and the use of manure and animal power for cultivation and transport.

In addition, the removal of the tsetse population from the Jozani forest reserve, where tsetse flies represented a major threat to adjacent livestock and agricultural systems, facilitated preserving this endangered habitat. Efficient wildlife management practices implemented after tsetse eradication even resulted in an increase in the numbers of some rare and protected wildlife species, such as the Zanzibar red colobus monkey, *Procolobus kirkii*.

For a long time, the tsetse and trypanosomosis problem did not get the attention it deserved, because it affects only rural Africa. The Zanzibar success raised the hope of African governments and stimulated similar campaigns on mainland Africa. At the African Summit of 2000, the Pan-African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) was established under the African Union Commission, calling for eradication of the tsetse from Africa over the next decades (Feldmann, 2004). In conjunction with technical support provided by the Joint FAO/IAEA Division, PATTEC has been supporting follow-up programmes that aim at integrating the

SIT for creating trypanosomosis- and tsetse-free zones in selected areas. Among the most advanced programmes are ongoing tsetse eradication campaigns in the Southern Rift Valley in Ethiopia and the Niayes region of Senegal.

CONCLUDING REMARKS

Success was the result of effective technology transfer and dedicated technical support by the FAO/IAEA, local and expatriate staff dedicated full-time to the campaign, training and commitment by the government and several donors. Other essential components of these area-wide eradication campaigns were: adequate public awareness and education; flexible management of the complex logistics; adequate baseline data collection; good sterile fly quality and back-ups for the fly colony; available spare parts for key equipment, and adequate infrastructure for the mass-rearing facility, including a gamma source and reliable access to electricity, water and other supplies. The isolated nature of Unguja Island and its distance of 35 km from the Tanzania mainland made it favourable to sustaining the status of eradication, as reinvasion was not a concern.

The eradication of tsetse from Unguja Island is not the only case of a successful AW-IPM programme with an SIT component. The New World screwworm fly, *Cochliomyia hominivorax*, which causes myiasis in warm-blooded vertebrates, including humans, livestock and wildlife, has also been successfully eradicated from all of North and Central America, as well as from an outbreak in Libya (Vargas-Terán, Hofmann and Tweddle, 2005). This pest is currently being contained with sterile males along the Panama-Colombia border. The SIT has been also very effective in suppression or eradication programmes against a number of major crop insect pests such as fruit flies and moths (Dyck, Hendrichs and Robinson, 2005).

To be able to move more decisively towards freeing larger areas in sub-Saharan Africa from tsetse, more capacity-building in all activities related to the area-wide implementation of the SIT will be required, as well as the establishment of a number of large tsetse fly mass-rearing facilities that should preferably be managed by the private sector.

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CHAPTER 4

CASE STUDIES IN THE FISHERY SECTOR





Application
of probiotics
using a
small boat
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CHAPTER 4.1

APPLICATION OF PROBIOTICS AS AN ENVIRONMENTAL TREATMENT AND FEED ADDITIVE IN THE PRODUCTION OF FARMED MARINE SHRIMP IN CHINA

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INTRODUCTION

Guangdong Province is the most important province for marine shrimp (*Penaeidae*) production in China. In 2010, it produced 400 000 tonnes of shrimp, which accounted for 30 percent of the total for the country. Since 1993, there have been numerous problems with shrimp farming in China relating to the deterioration of the environment and the occurrence of disease. With the ban on using antibiotics in aquaculture, scientists and farmers have tried to find alternative methods to improve the aquaculture environment, introduce new species for culture and improve growth performance.

Commercial shrimp aquaculture production in China is characterized by high stocking density, intensive management and high economic returns. These practices also lead to many problems in shrimp ponds, such as overfeeding, high organic content, and changes in the micro-organism ecosystem. These changes can lead to eutrophication, water quality deterioration and a reduction in shrimp growth.

To control and prevent diseases, chemicals and antibiotics were used in shrimp production. These drugs have proved effective in fighting disease outbreaks, but have also caused problems, such as drug residues in shrimp, water pollution, lower productivities and destruction of the ecological food chain. In 2002, Chinese processed shrimp products were banned for export to the European Union market because of antibiotic residues in shrimp meat (this ban was lifted in 2004). To meet the demand for environmentally friendly aquaculture practices, an alternative to antibiotic use in aquaculture was adopted. The use of probiotics was one such alternative.

In China, the first probiotic strain for aquaculture was reported by the Sichuan Agricultural University in 1995. By 2001, the Ministry of Agriculture had approved 13 probiotic strains for agriculture practices. Now, there are more than 400 companies producing *Bacillaceae*, yeast, *Rhodospirillaceae* and *Lactobacillus* probiotics for agriculture, with an annual production of 10 000 tonnes. Approximately 2 000 tonnes of probiotics are used in Chinese aquaculture. None are exported from China. Numerous research academies and universities tried to select more efficient and specific probiotic strains for use in aquaculture. Many companies have already developed their own probiotic strains and are trying to produce enough to satisfy the market demand. In 2011, there was a reported demand for 30 000 tonnes of probiotic products in China for aquaculture practices. After decades of research and development, the use of probiotics is now popular in the commercial production of farmed shrimp.

PROBIOTICS IN SHRIMP FARMING

As an alternative strategy to antibiotic use in aquatic disease management, probiotics have attracted extensive attention. In 1998, a reported application of a probiotic, *Bacillus* spp., in shrimp ponds led to higher survival rates of shrimp that had been infected by *Vibrio* bacteria, and further successful cases were reported thereafter. The probiotic is used to improve the water quality, as an additive to feed, and to improve disease prevention. It is applied in the nursery pond and grow-out pond; it can be used in different forms, such as powder, liquid or as additives to pellet feed. The probiotics improved the water quality as well as the survival rate and growth of the shrimp.

Other bacteria that have been used successfully as probiotics belonging to the genus *Vibrio*, *Pseudomonas* and *Bacillus*, and the species *Thalassobacter utilis*. Most researchers have isolated these probiotic strains from water bodies used for shrimp culture, or from the intestine of different *Penaeid* shrimp species. The use of probiotic bacteria, that outcompete and displace harmful bacteria, and the use of immune-stimulants are two of the most promising preventive methods developed in the fight against shrimp diseases. Probiotic bacteria also produce some digestive enzymes that might improve the digestive system, enhance stress resistance and improve the health of the shrimp.

The use of probiotics in aquaculture has become a promising means of balancing the micro-environment in shrimp ponds in order to improve productivity and the health of the water in an ecologically friendly manner. The practice has attracted increasing attention from aquaculture extension officers, farmers and environmentalists.

Three bacterial genera, *Bacillus*, *Vibrio* and *Pseudomonas*, are commonly administered as probiotics in shrimp aquaculture. Candidate probiotics are species-specific and need to be tested for their effectiveness for certain species *in vitro* and *in vivo*. A solution containing the appropriate probiotic can be used for pond disinfection and water quality improvement before filling the pond with water. Water quality can also be improved by administering the probiotic to ponds a few days before stocking with shrimp. For improvements in shrimp growth, it is best to add the probiotic directly to the shrimp feed and several techniques are used to accomplish this.

Overdosage or prolonged administration of probiotics can induce immune-suppression in shrimp. Therefore, a combination of probiotics results in better outcomes for the shrimp than using individual probiotics.

TECHNOLOGY TRANSFER

The use of probiotics was promoted through several methods. Because shrimp farming is an expensive business to operate, many farmers tried to find solutions to recover shrimp farming costs. With the support of the extension systems in China and commercial production of probiotics, the farmers can easily access the products from the market. The technical assistance on how to use probiotics was provided by the local fishery technical extension officer, and retail sale support by private companies and sales outlets. The fishery technical extension station in the province selected demonstration farms and farmers to use the probiotics and train additional farmers on the use of probiotics. The sales company promoted the probiotics to shrimp farmers by providing technical advice and extension for a better understanding of how to use probiotics in aquaculture. Farmers used probiotics as an alternative to antibiotics to fight the daily problems in water treatment and shrimp mortality. The ease of accessing training and the positive results achieved in the production of shrimp led to the increased adoption of probiotics.

There are three approaches to the financing of the application of probiotics to aquaculture.

- i) Demonstration project: demonstration farms were selected and funded by the government through the fishery technical extension station. The farms would then receive intensive technical guidance and initial funds to carry out the application of probiotics to their own shrimp ponds.
- ii) The sales company provided free trial probiotic liquids to demonstration farmers, and set up successful examples to advertise their products.
- iii) Some farmers purchased the probiotic liquids and carried out pilot projects on their farms before applying them for the whole farm. When they succeeded in the application, they continued to finance the use of probiotics themselves and applied the new technology in their farm as part of a sustainable aquaculture business.

The research academies and universities were the major organizations in technology innovation. They had advanced research facilities and developed many probiotic strains for use in aquaculture. Commercial companies used to cooperate with research academies and universities in new technology development. The research achievements and products could be transferred to company for commercial production, through contracts or agreement. There are also some imported products, such as probiotics from Japan, Sweden and the United States. There is need for authorized sales permission or agreement between the companies.

From left to right
Preparation of
probiotics before
application to the
pond

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Application of
probiotics using a
foam board

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INTELLECTUAL PROPERTY RIGHTS ISSUES

There is the potential for intellectual property rights to be applied to some specific strains of probiotics, culture methods and the formula of culture medium. There are reports of patented probiotic strains and culture methods in China, but there are no reports on the use of these patents for actual products in current aquaculture practices. The development of commercial probiotic products has involved research by private industry or cooperation between private industry and research academies, and therefore represents a public-private partnership. Intellectual property rights would need to address this aspect. In future, specific and effective probiotic products with patents should be encouraged.

CHALLENGES ENCOUNTERED IN USE OF PROBIOTICS

The challenges of using live organisms such as probiotics in treating aquaculture species, feed and ponds included:

- i) The success of probiotics in water strongly depends on their density and activities. Therefore there is a need to monitor and adjust the density of the probiotics and their activities in water during the application.
- ii) Some of the strains used in aquaculture were isolated from fish ponds or fish intestines, while others were developed for use with poultry. Therefore, there is a need to develop specific probiotic strains for other aquatic species.
- iii) There is a need to carry out more research to improve understanding of the mechanisms of how probiotics function in the organisms and in aquaculture ponds.
- iv) The production, processing, transportation and storage of probiotics should be developed for higher efficiency.
- v) The advantages and disadvantages of using single strains of probiotics or combinations of strains should be studied to improve efficiency.

- vi) Policies and regulations need to be formulated to increase the distribution of probiotics. The regulations and policies to use probiotics in different countries are not the same. Most countries regulate probiotics as micro-organisms and do not permit importation because of environmental risk considerations. Permission to import also may experience delays due to the time required for the safety inspection.

IMPACT AND LESSONS LEARNED

Shrimp farmers in China are business-oriented; farmers pay rent to the government for the use of ponds. The application of probiotics became an important and common approach in ensuring adequate production and profit from the ponds both for small-scale (ponds of < 1 ha) and for large scale (ponds > 1 ha) commercial shrimp farmers. There are many reports on the successful application of probiotics in shrimp farming. The growth of the shrimp postlarvae (PL) was 17 percent higher than the control group and the survival rate improved 111 percent. The water quality improvement results were also very good, the ammonia nitrogen was reduced by 60 percent, and nitrate by 87 percent. Toxic matters were removed and the survival rate of PL and shrimp was greatly improved. After 85 days of farming, the yield of the probiotic-treated group was 227kg/667m² (or about 3.4 t/ha), almost double the yield of the control group. Additionally, the individual size of the treated group of shrimp was larger than the control group.

Probiotics are useful micro-organisms for improving the efficiency of aquaculture production in an environmentally friendly manner. Although there were many reports on the successful application of probiotics in aquaculture, future research and development projects are still needed to understand exactly how probiotics function.

- i) The research should be based on the local farming situation, with the aim of solving the problems in sustainable production.
- ii) Research academies and universities, along with private industry, have played important roles in research and development. However, government support is still needed.
- iii) Commercial production, private industry and extension can be very effective at promoting technology exchange and adoption.

Adoption of the new technology was strongly assisted by the demand for high-quality shrimp and sustainable aquaculture practices. As an alternative to antibiotics use in aquaculture, probiotics helped in the recovery in the production of farmed shrimp, and allowed farmed shrimp to continue to be an important export species. In light of ongoing requests by the government, trade companies and producers, more probiotic strains will certainly contribute to future good harvests, sustainable production and strong economic returns.



Distribution of shrimp postlarvae among society farmers
©Arun P. Padiyar

CHAPTER 4.2

PCR-BASED PATHOGEN DETECTION IN SHRIMP AQUACULTURE IN INDIA

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SHRIMP AQUACULTURE AND DISEASE SCENARIO IN INDIA

Shrimp farming continues to be the largest export-oriented aquaculture production sector in India, providing a significant contribution to the world's farmed shrimp production. The majority of shrimp farming in India is by low-income small-scale farmers (Umesh *et al.*, 2010). Therefore, properly managed farming offers significant employment opportunities and helps alleviate poverty among the coastal population. Although there are many species of shrimps, only a few of the larger ones are cultivated in India. The two species currently dominating the Indian shrimp farming are: Giant Tiger Shrimp, *Penaeus monodon* (*P. monodon*), and the Pacific White Shrimp, *Penaeus vannamei* (*P. vannamei*). Intensification of shrimp farming has caused many shrimp diseases of epidemic proportion over the last two decades, particularly viral diseases in the densely populated monoculture farms. Such virus infections spread rapidly and cause massive crop losses in clustered farming, directly impacting the income of farmers (Walker & Mohan, 2009). Thus, 'better management practices' (BMPs) are immensely important to the success and sustainability of the shrimp industry (Mohan *et al.*, 2008; Padiyar, 2005a; Subasinghe, 2005).

Viral pathogens reported in Indian shrimp farms and hatcheries are: white spot syndrome virus (WSSV), yellow head virus, Monodon baculovirus (MBV), Hepatopancreatic parvovirus (HPV), Laem-Singh virus and *Macrobrachium rosenbergii* nodavirus (MrNV) (Prakasha *et al.*, 2007; Ravi *et al.*, 2009; Walker & Mohan, 2009). Bacterial diseases, such as luminescent vibriosis, caused by *Vibrio spp.*, are also sporadically reported as affecting shrimp farms.

In India, by far the major economic losses are caused by white spot disease (WSD) epidemics, caused by WSSV, which is the most devastating pathogen due to its wide host range and ability to cause 100 percent mortalities within days. Epidemiological studies suggest high prevalence of WSSV among the shrimp broodstock and postlarvae (PL) populations (Corsin *et al.*, 2003; Otta *et al.*, 1999; Thakur *et al.*, 2002). Stocking infected PL remains a major source of WSSV infection in the culture systems, although the virus can be effectively transmitted horizontally by water-borne contact or ingestion (Lo *et al.*, 1998). BMPs developed from epidemiological studies recommend stocking pathogen-free PLs to minimize the risk of outbreak and economic loss (MPEDA/NACA, 2001). Instead of using larvae from wild catches, specific pathogen-free (SPF) brood stocks raised in captivity are being used in an effort to reduce disease risks. In India, *P. monodon* used to be the major farming species until recently. However, because of the susceptibility to WSD due to non-availability of SPF stocks, it is being gradually replaced by *P. vannamei* since 2008, as one of the measures of reducing losses in shrimp farming. Since 2010, the majority of the shrimp farmers in India have switched to *P. vannamei* culture.

ADVANCES IN PCR AS A BIOTECHNOLOGICAL TOOL FOR SHRIMP DISEASE MANAGEMENT IN INDIA

Biotechnological tools of relevance to shrimp disease management have been applied for better prevention strategies, routine pathogen screening and to develop specific disease resistant strains. Currently, these technologies are used in the Indian shrimp industry at various levels, depending on the intensity and commerciality of the farming system.

With the high prevalence of viral infections in broodstock and PL populations (Corsin *et al.*, 2003; Thakur *et al.*, 2002), and the fact that other crustaceans can be carriers of the virus, the most sought after biotechnological tool in shrimp farming is an effective means of pathogen screening. During the early 1990s, no techniques were available to screen effectively for pathogens. However, with the advancement of biotechnological research, there are now many DNA-based detection technologies, including polymerase chain reaction (PCR)-based diagnostics available for all the major shrimp viruses. A number of PCR tests have been developed for the detection of the prevalent viruses in shrimp farms (OIE, 2012). Similarly, using nested PCR and dot-blot hybridization techniques, viral prevalence could be detected in wild shrimp species in Indian coastal waters (Manjanaik *et al.*, 2005). Multiple viral infections in apparently healthy PL samples could be effectively detected using PCR methods specific to MBV, HPV and WSSV (Manivannan *et al.*, 2002). These PCR tests use a set of different primers targeted to amplify specific regions of each of the virus genomes. Several reverse transcriptase PCR (RT-PCR) tests are also available to detect RNA viruses. Similarly, PCR tests have also been developed to detect bacterial pathogens such as *Vibrio* species (Karunasagar, 2000). Recently, rapid and cost-effective immunodiagnostic strips have begun to appear for screening shrimp diseases (Patil *et al.*, 2008). Advanced methods such as multiplex PCR and pond side diagnostic tools such as loop-mediated isothermal amplification (LAMP) and insulated isothermal PCR (iiPCR) are also now available for single or multiple viral detections. Using these biotechnological tools, unskilled farm personnel could be trained to diagnose shrimp disease outbreaks at the farm.

RECOMMENDED AND COMMONLY USED PCR DETECTION METHODS IN INDIA

PCR-based pathogen detection methods in shrimp farming were commercially introduced in India in 1996 by the Marine Products Export Development Authority (MPEDA), fisheries

research institutes and various private agencies. During the initial years, farmers used to screen for MBV, HPV and infectious hypodermic and haematopoeitic necrosis virus aside from WSSV. However, since it became apparent that the crop losses were primarily attributed to WSD, farmers resorted to routine PCR screening of WSSV in broodstock and PL in hatcheries and farms as one of the most common health management strategies.

The best life stages of crustaceans for PCR detection of WSSV are late PL stages, juveniles and adults. Persistent infection occurs commonly and lifelong infection has been shown. In such cases, probability of detection can be increased by exposure to physiological or environmental stress [Joseph & Philip, 2007; Vidal *et al.*, 2001]. Since the major WSSV target tissues are of ectodermal and mesodermal origin [Lo *et al.*, 1997], the pleopods (swimming legs) are most commonly used for PCR screening as a non-invasive method for adult shrimps. The dead and moribund samples from the culture ponds can also be effectively used for PCR tests, as they often provide indication of a disease outbreak [Mohan *et al.*, 2002]. Similarly, epidemiological investigations reveal that PCR screening of shrimp samples during middle of the culture period may serve as an indicator of crop status and help in predicting disease outbreaks and optimizing harvest strategies [Sahoo *et al.*, 2010; Turnbull *et al.*, 2005]. The PCR protocol developed by Lo *et al.* [1996, 1997] is recommended for WSSV diagnosis in most situations [OIE, 2012]. A 1st-step PCR-positive result using this method indicates a serious WSSV infection, whereas, a 2nd-step nested PCR-positive result may indicate a latent or carrier-state infection. For PL or broodstock screening, a 2nd-step nested PCR is necessary for the detection of carrier status. Two-step PCR and sequencing are the recommended methods for declaring WSSV-free status, although extremely low viral loads during latent infection are sometimes undetectable even by sensitive methods such as real-time and nested PCR.

Several commercial PCR kits are now available for WSSV diagnosis and are acceptable provided they have been validated. OIE guidelines are used to bring uniformity in PCR screening practice and the OIE Register may be consulted for certified kits. Currently, there are four PCR detection kits available for shrimp pathogen screening in the Indian market: IQ2000 WSSV Kit (GeneReach, Taiwan), Bangalore Genei kit, Mangalore Biotech kit and Poseidon Biotech kit. However, it is important to adhere to uniform standards, recommended quality control measures with appropriate positive and negative controls during PCR detection to reduce chances of false positives and false negatives leading to confusion among the farmers about which method to use. With the knowledge of various geographical isolates of WSSV with genotypic variability [Marks *et al.*, 2004; Walker *et al.*, 2011a], DNA-based diagnostic methods such as PCR requires rigorous standardization to detect all the virulent strains.

from left to right
Farm site
collection and
preservation of
shrimp samples
for PCR test
©Arun P. Padiyar



P. monodon
broodstock in
hatchery
©Arun P. Padiyar



POPULARIZATION OF PCR TO SCREEN SHRIMP PATHOGENS IN INDIA AND ITS EFFICACY IN CONTROLLING DISEASE OUTBREAKS AND CROP LOSSES

Over the past decade, PCR-based rapid detection of shrimp pathogens has given a new dimension to the Indian shrimp industry. Beginning in 2002, MPEDA promoted the establishment of PCR labs by offering subsidies. About 45 private entrepreneurs and corporate entities started PCR diagnostic labs in Andhra Pradesh and Tamil Nadu, where most of the shrimp hatcheries were located. Subsequently, with the involvement of the Indian Council of Agricultural Research (ICAR), Australian Centre for International Agricultural Research (ACIAR), Network of Aquaculture Centres in Asia-Pacific (NACA) and FAO collaborations, MPEDA made PCR labs mandatory for *P. monodon* hatchery registration and offered required training and resources. This subsequently increased the popularity and accessibility of PCR among the small-scale farmers. As a result, there are now more than 100 hatcheries with on-site PCR labs to screen broodstock and PL. This initiative also facilitated increasing awareness among farmers of PCR screening as a method of choice. As the usage of PCR diagnostics increased, the retail cost of PCR tests significantly decreased, making it more affordable to the small-scale farmers.

In the pilot projects on the Indian east coast conducted by MPEDA/NACA and FAO, the use of PCR screening was the crucial BMP for the small-scale shrimp farming in Andhra Pradesh. The MPEDA/NACA project in collaboration with FAO and ACIAR, initiated during 2000 to support small-scale shrimp farmers in disease management, resulted in the increased usage of PCR screening. Contract hatchery systems promoted by the MPEDA/NACA project helped farmer societies/aquaclubs to procure large quantities of PCR-screened PL with improved field-level management efficiencies, providing significant improvements in profits and reduced risk of disease outbreaks (Padiyar *et al.*, 2003, 2005b). An economic analysis of the farmer groups in Andhra Pradesh indicated that farmers adopting BMPs, including the use of PCR screening

of PL, had better profits and were able to produce quality crops (Umesh *et al.*, 2010). Such participatory projects have helped disseminate the PCR techniques among farmers and resulted in increased participation, with more than 10 000 farmers now active in about 150 aquaculture societies in five coastal states of India. Since 2007, the MPEDA/NACA collaborative project became the National Center for Sustainable Aquaculture (NaCSA), which serves as an outreach organization of MPEDA for the small-scale aquaculture sector, providing technical support to farmer groups to enhance shrimp production in a sustainable and profitable manner. The success is built on reduction of losses from disease outbreaks in production systems, and to a large extent this has been made possible by PCR technology for screening and detecting major viral pathogens in broodstock and PL.

One of the recent trends of Indian shrimp farming sector is the introduction of *P. vannamei* and its increasing popularity among shrimp farmers. This has culminated in conversion of the majority of *P. monodon* farms into *P. vannamei* farming. Although this may be attributed to the relatively faster growth rate and lower cost of production of *P. vannamei*, the availability and sourcing of SPF stocks of *P. vannamei* has also contributed to this preferential change among farmers. Since large-scale commercial introduction of *P. vannamei* in 2009, many private PCR labs reported decline of their business, because farmers were given to understand that PCR tests are not needed for *P. vannamei* SPF stocks. However, MPEDA and the Coastal Aquaculture Authority now make it mandatory for all the *P. vannamei* licensed hatcheries to have an in-house PCR lab and test for all the OIE listed shrimp pathogens on the SPF broodstock and PL batches. Currently, the imported *P. vannamei* broodstock are quarantined at the Aquatic Quarantine Center of the Rajiv Gandhi Center for Aquaculture and screened for OIE listed pathogens by PCR. Then the stocks are released to importing hatcheries after confirmation of freedom from the listed pathogen.

Though PCR proved to be a helpful tool to contain the diseases at farm and cluster levels where it was used as one of the BMPs, there was stagnancy in shrimp production during 2005-2009. The authors feel that such stagnation may be attributed to: (1) PCR was not used in the right place. *P. monodon* brooders from the wild were rarely tested by hatcheries, and the PCR test was restricted to only PL by farmers, (2) large numbers of open farming system with very loose biosecurity, increasing the risk of horizontal infections in the culture areas, (3) poor farmer cooperation and farming discipline at farm cluster levels, especially among the small-scale farmers.

Crop loss due to disease outbreaks in some states, e.g. Andhra Pradesh, Tamil Nadu and Gujarat, were significantly reduced due to high level of awareness about recommended PCR screening methods and BMPs among farmers. The prevalence of disease outbreak among farmer societies

promoted by NaCSA has come down to 20 percent from a baseline of 80 percent prior to BMP implementation (Umesh *et al.*, 2010). There was a significant increase in national shrimp production from 2008 to 2012 (MPEDA, 2012). This may largely be attributed to the use of *P. vannamei* SPF stocks and a strong quarantine system which uses PCR to screen the imported SPF animals. From analysis of the current trends, and through the first-hand information from our farm-level questionnaires, we reason that PCR is an important health management tool for preventing crop losses due to viral disease outbreaks. However, proper use of the technology and the compliance of farmer/hatchery owners to adopt PCR screening and BMPs is equally important to gain benefits from these biotechnological applications in the shrimp farming industry.

FUTURE CHALLENGES AND EMERGING PATHOGENS

Shrimp aquaculture in India is continuously evolving and new species have been introduced, which increases the chances of new pathogens and disease outbreaks. For instance, recent crop losses due to distinctive pattern of mortalities in *P. monodon* and *P. vannamei* farms of China and Southeast Asia, attributed to early mortality syndrome or acute hepatopancreatic necrosis syndrome, indicates plausible presence of new pathogens, although the causative agent is not yet known and the disease is still described as idiopathic (NACA, 2013). There have been reports of emerging viral infections caused by MrNV and extra small viruses (XSV) in *M. rosenbergii* and *P. indicus* PL batches in Indian hatcheries and PCR techniques are being developed and popularized for detection of such emerging pathogens (Ravi *et al.*, 2009). Realizing the loss in freshwater prawn at early stage due to MrNV, an OIE reference laboratory has been set up in Tamil Nadu for routine screening by RT-PCR. In addition, LAMP and multiplex RT-PCR methods have been developed (Haridas *et al.*, 2010; Pillai *et al.*, 2006). However, PCR screening for such emerging pathogens is rarely practised and farmers should be made aware of potential new pathogens and proper biosecurity measures to control future outbreaks. Finally, despite using PCR-screened broodstock and PL, WSSV infection and disease frequently occur through various horizontal and poorly understood dynamics of infection modes in the culture system (Walker *et al.*, 2011a, b). Therefore, juveniles and adults in the culture ponds, as well as other potential horizontal sources such as crustaceans in the pond area and feed, should be periodically monitored for WSSV contamination during the culture period.

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from top left,
clockwise

Farmers
harvesting the
hybrid catfish
@Sathid Chatchaiphan

A farmer
sacrificing a male
African catfish to
collect testes
@Sathid Chatchaiphan

A farmer preparing
sperm solution of
African catfish
@Sathid Chatchaiphan

A farmer
squeezing
eggs from the
hormone-injected
female
@Sathid Chatchaiphan

A farmer grading
the hybrid after
harvesting
@Sathid Chatchaiphan

CHAPTER 4.3

INTERSPECIFIC HYBRID CATFISH IN THAILAND

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Interspecific hybridization is an approach to producing living organisms that rarely exist in nature. The general objective is to combine desirable traits from maternal and paternal species, to make use of hybrid vigour or heterosis (a phenomenon where the hybrid performs better than the average of the parental species).

Interspecific hybridization occurs more easily in fish than other vertebrates, probably because of the flexible sex-determining system of fish, which enables the better survival of the hybrids. Although interspecific hybridization has been done in laboratories, very few hybrids have been used at a commercial scale. However the hybrid between the broad-head catfish, *Clarias macrocephalus* and male African sharp-tooth catfish, *Clarias gariepinus* is a good example of a commercially successful hybrid.

REASONS FOR THE HYBRIDIZATION BETWEEN THE BROAD-HEAD CATFISH AND AFRICAN SHARP-TOOTH CATFISH

The broad-head catfish is native to Southeast Asia. It is found in shallow freshwater throughout the region. It is a favourite food fish in Thailand and the neighbouring countries because of its desirable yellowish colour and the texture of its flesh, and it thus commands a relatively high market price. However, the culture of this species was not successful on a commercial scale due to its slow growth that required at least seven months reaching a marketable size of 200 grams. Moreover, it is very susceptible to bacterial diseases that reduced the survival rate to about 30 percent. These were the main reasons for hybridizing the broad-head catfish with the African sharp-tooth catfish, *C. gariepinus* which was introduced from Vietnam via Laos in 1987 (FishBase, 2007).

The African sharp-tooth catfish is native to Africa. It was introduced to Southeast Asia because of its high growth rate (reaching 200 grams body weight within 3 months) and low mortality due to diseases (survival rate about 80-95 percent). However, its meat quality (white and soft meat) was not well accepted by local people. Therefore, it was hybridized with some local catfish species in the hope of producing fast-growing, disease-resistant hybrids with acceptable meat quality.

ESTABLISHMENT OF THE TECHNOLOGY

There has been no record of the first hybridization attempt between these two species. Anecdotal reports state it was first done by a local farmer in northeastern Thailand by trial and error. The technology has been rapidly transferred among catfish hatcheries because the protocols were similar to the well-known breeding practice of the broad-head catfish. In brief, the hybridization is done by artificial insemination where a mature female broad-head catfish is injected once with a hormone (LH-RH analogue, Trade name: Suprefact) at 25 µg/kg plus Domperidone at 5 mg/kg while the male African sharp-tooth catfish is injected with the same hormone at 10 µg/kg and the same dosage of Domperidone. About 15 hours after injection the females are made to release their eggs by the breeder gently pressing on the belly. The eggs come out easily through the urogenital opening. Then the eggs are mixed with sperm solution squeezed from the minced testes of the sacrificed male. The sticky fertilized eggs are simply spread on the fine mesh net immersed under water. Hatching occurs 24-36 hours after fertilization. The fry are then fed with live food (water fleas) and will reach fingerling size of 1 inch within 10 days. The hybrid is very similar to the broad-head catfish and is always misidentified by local people as this species.

Nukwan *et al.* (1990) were the first group to perform scientific investigations on this hybridization. They found that only the hybrid having the broad-head catfish as the mother hatched and survived well; the hybrid did not show heterosis for growth (i.e. the growth rate of the hybrid was not higher than average growth rate of the parents). The advantages of the hybrid over the maternal species (the broad-head catfish) was the improved growth rate (reaching 200 grams body weight within 4 months) and survival rate, while the meat quality was better accepted than that of the paternal species (the African catfish).

CONTRIBUTIONS OF THE HYBRID CATFISH

The hybrid has been widely accepted by the farmers and as such, it enhanced the country's annual production of walking catfish (mixed species of the genus *Clarias* are often generally referred to as walking catfish) from 17 900 metric tonnes in 1990 to the peak of 159 314 metric tonnes in 2004 despite a slight decline during the recent years (Figure 1).

The beneficiaries of this technology are the catfish hatcheries and the grow-out farmers. The catfish hatcheries that practise the hybridization technology are located mainly in central

Thailand where the water flea *Moina macrocopa*, an essential food for the fry, is commercially available. Although the exact number is not known, the number of hatcheries is estimated to be at least 1 000 throughout the country. The number of grow-out farms is much higher. In 2010, there were 79 288 catfish farms in Thailand covering an area of 14 432 hectares (Research and Statistical Analysis Division, 2010). At present, among freshwater commodities in Thailand, hybrid catfish production is second only to Nile tilapia, and Thailand has been ranked first among the walking catfish producing countries (FAO, 2010). This has made this technology a successful case study of the application of biotechnology to aquaculture.

The culture of the hybrid has been practised at either commercial or backyard scale. The commercial farms stocked the hybrid in earthen ponds of varying sizes (large ponds of more than one hectare in the central provinces and small ponds in northeastern provinces). The stocking density of the 2-cm long fingerlings is very high (about 1 000 000 fingerlings per hectare). The culture period is about 4-5 months and the yield is about 70 000 kg/hectare in the central provinces, slightly lower in the northeastern provinces. The survival rate ranges from 40 to 60 percent. Feeding differs according to parts of the country. For example, the farms in central Thailand have access to cheap, high nutritional value wastes (e.g. chicken viscera, waste from fish processing plants), thus they rely on these wastes. The additional reason is the low market price of the hybrid catfish in central Thailand (US\$0.8/kg) that forces the farmers to reduce the cost. In northeastern provinces where the hybrid demands higher market price (e.g. US\$1/kg), farmers use commercially available pellet feed only.

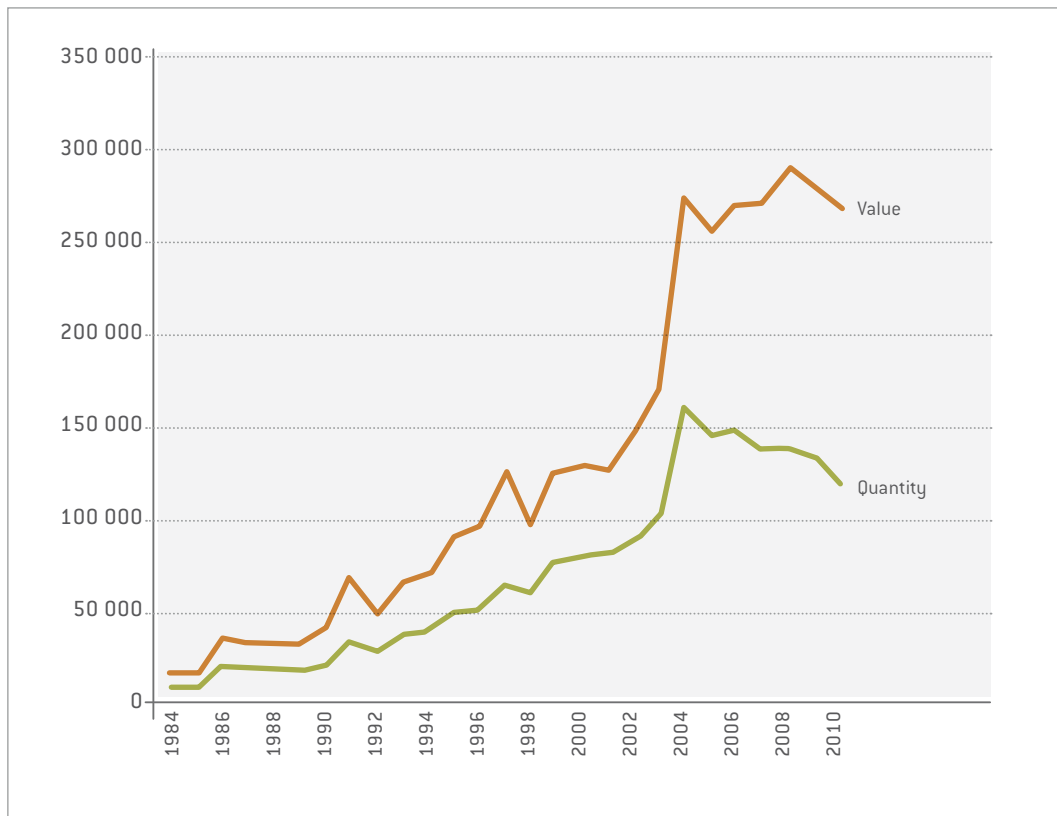
The backyard culture of the hybrid catfish is practised in rural areas and the production does not significantly contribute to the country's production. However, it does provide poor people with a cheap protein source and small income from limited resources available. The fingerlings (mostly of large size about two inches) are stocked in small ponds (100-200 square metres), tanks or cages, at relatively low stocking density. They are fed mainly with commercially available pellets while locally available protein sources (e.g. silk worm pupae, termites) are occasionally used as feed whenever available. The culture period is about three months when the 100-gram fish are harvested. At present, backyard culture is being promoted throughout the country as a part of a drive for self-sufficiency in agriculture.

CURRENT PRODUCTION TREND

As previously mentioned, the country's annual production of walking catfish has declined slightly. The major obstacle is the reduction of its market price. Since the export market of catfish is very limited, a majority of the hybrid catfish production is consumed domestically.

As such, the market is easily oversupplied, which affects the market price. In addition, deteriorating pond environments are triggering disease outbreaks with increasing frequency. These are the major causes of the recent reduction in annual production.

Figure 1. Quantity (metric tonne) and value (US\$) of hybrid catfish annual production during 1984-2010



Source: FAO (2010)

ADVERSE IMPACTS OF THE HYBRID CATFISH

A dark side of the practice of hybridization also exists. Despite the fact that a majority of the interspecific hybrid animals are sterile, the female hybrid catfish produces mature eggs although in numbers far lower than the parental species; the males show a higher degree of sterility. As such, there was a concern that if the hybrid escaped into natural water bodies, it might backcross with the native catfish species including the broad-head catfish. The repeated back-crossing could eventually result in genetic introgression which may compromise the fitness of the local species.

During the past decades, Thailand has frequently faced severe floods, which resulted in the escape of millions of the hybrids into natural water bodies. The adverse impact of the escapees was revealed by the detection of gene-products in the wild broad-head catfish collected from habitats in every part of the country (Senanan *et al.*, 2004; Na-Nakorn *et al.*, 2004) that were found previously only in the African sharp-tooth catfish. This supports the hypothesis that the escaped hybrid may be contributing to the decline of native walking catfish by reducing their fitness. However, other factors such as habitat loss and overharvesting would also contribute to the decline of the native catfish. So far, there are no measures to mitigate the problem of genetic introgression, while the escape of the hybrid from grow-out farms still occasionally occurs.

Moreover, the expansion of the hybrid culture has adversely impacted the environment through the release of aquaculture waste. The grow-out ponds are always stocked with the hybrids at very high density and fed with high-protein feed. Therefore, wastewater from catfish farms is heavily loaded with organic matter and rich in nutrients. As such, when it is released to natural waters it causes rapid depletion of dissolved oxygen and thus causes mass mortality of aquatic animals. The wastewater also interrupts the flowering of rice due to excess nitrogen.

CONCLUSION

Overall, the hybridization technology has made a highly significant contribution to the aquaculture production of walking catfish in Thailand. The technology has triggered enormous expansion of the aquaculture business of walking catfish, feed industries and other related businesses. Moreover, it enhances the access of poor rural people to cheap and high-quality protein from the hybrid. Nevertheless, the farming of hybrids also has an adverse impact on native species and the environment. The adoption of this technology in the future should therefore be accompanied by an awareness of the adverse impacts. Good guidelines for the breeding of the hybrid should be established and endorsed for the sake of the ecological sustainability of aquaculture.

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Farmer catching the market-sized Jian carp from the cage
©Zaijie Dong

next page
Farmers harvesting Jian carp from the pond for sale
©Zaijie Dong

CHAPTER 4.4

USE OF WITHIN-FAMILY SELECTION AND GYNOGENESIS TO DEVELOP THE JIAN CARP (*CYPRINUS CARPIO* VAR. *JIAN*) IN CHINA

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INTRODUCTION

Heterosis, also known as hybrid vigour or outbreeding enhancement, is the improved or increased function of any biological quality in hybrid offspring. Heterosis has been used as an effective way to improve fish quality and increase fish production. Since the 1970s, Chinese fishery scientists have made broad studies of the utilization of heterosis in the production of common carp (*Cyprinus carpio*) and have made great achievements. Some hybrids of common carp have been successfully used in aquaculture. Heyuan carp is one hybrid which is obtained from the cross between purse red carp (*C. carpio* var. *wuyuanensis*) ♀ × Yuanjiang carp (*C. carpio* var. *yuankangensis*) ♂. It has several advantages, including high growth rate, good body shape, good feed conversion rate and high seinability (easily collected by nets in ponds). To produce Heyuan carp, pure populations of purse red carp and Yuanjiang carp must be maintained and the sexes in both populations chosen carefully during mating. The resulting Heyuan hybrid cannot be used as brood fish because the traits will segregate when the hybrids are mated. How to fix the hybrid vigour in the brood fish, i.e. pass on the same combination of desired traits in the offspring, and make the mating of brood fish easier for farmers is very important for smallholder farmers to produce quality seeds for aquaculture.

APPROACH USED: THE MATING PROGRAMME

Funded by projects from the Chinese Government, scientists at the Freshwater Fisheries Research Center of the Chinese Academy of Fishery Sciences made efforts to develop a population of carp that would allow farmers to utilize the heterosis of Heyuan carp by means of an easy way of crossing males and females in the population. The methodology of combining within-family selection and chromosome engineering (gynogenesis) was adopted to increase the homozygosity of the loci controlling the desired traits. The detailed mating programme (Figure 1) is summarized below.

Step 1: establish four families. Choose four female purse red carp and four male Yuanjiang carp, cross purse red carp ♀ × Yuanjiang carp ♂ to produce the first filial generation (F1) of four hybrid families.

Step 2: conduct within-family selection. Select males and females with good traits for fast growth, grey colour and long body shape from the F1 generation in each of the four families. Cross the males with females from the same family to produce the second filial generation (F2) of four families.

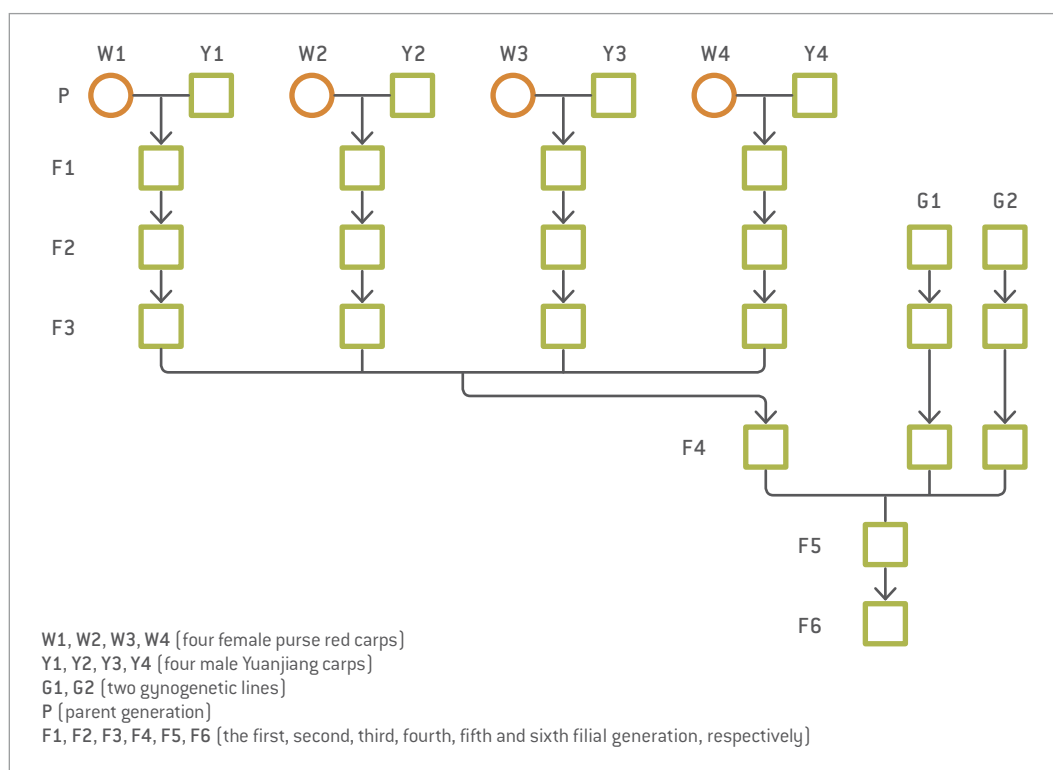
Step 3: conduct within-family selection. Select males and females with good traits from the F2 generation in each of the four families. Cross the males with females from the same family to produce the third filial generation (F3) of four families. Meanwhile, conduct gynogenesis. (Gynogenesis is a special form of sexual reproduction in which insemination is necessary but the head of the sperm penetrating into the ovum does not transform into male pronucleus; and the gynogenetic embryo develops at the expense of the ovum nucleus only, i.e. the male gamete contributes no genetic material to the embryo. Consequently the gynogenetic offspring are all females, identical to the mother). Select two good females based on the growth, colour and body shape. Strip matured eggs from the two fish and inseminate them with inactivated sperm. Induce two lines of gynogenetic fish by cold shock treatment at 0-2°C (G1 and G2).

Step 4: conduct within-family selection and gynogenesis. Select and merge males and females with good traits from all the F3 families, they randomly mate with each other to produce the fourth filial generation (F4). Meanwhile, select one good female fish in each of the two gynogenetic lines, induce the second generation of two gynogenetic lines (boxes below G1 and G2).

Step 5: conduct random mating. Select good fish from the F4, as well as select good females from the second generation of two gynogenetic lines. Merge them together and mate randomly to produce the fifth filial generation (F5).

Step 6: conduct random mating. Select good fish from F5, and randomly mate them to produce the sixth filial generation (F6).

In F6, the traits are stable, more than 95 percent of fish were grey in colour and had a long body shape (ratio of standard length to body depth was 2.68 ± 0.38). From this generation onward, the fish were officially designated as Jian carp (*Cyprinus carpio* var. *jian*).

Figure 1. Schematic diagram of Jian carp breeding method

DIFFERENCE MADE

After six generations of selection for growth, colour and shape, Jian carp is a good stock of cultivated fish species with stable genetic traits for grey colour and long body shape. The growth of Jian carp outperformed its original parents and their hybridized filial generation, i.e. purple red carp, Yuanjiang carp and Heyuan carp, by a factor of 141-250 percent, 80-96 percent and 40-42 percent respectively. More importantly, the male and female individuals in the population could be easily mated by farmers to perpetuate the strain. Jian carp was first distributed to farmers in 1988. At present, Jian carp has been distributed to 27 provinces, municipalities or autonomous regions in China with great social and economic benefits. Approximately 160 000 farms use the Jian carp, many of which are smallholder farms. The specific yield of this carp is more than 30 percent greater than other varieties of common carp and the production of Jian carp is 1.35 million MT and accounts for more than 50 percent of total common carp production with an approximate value of 12.8 billion RMB.

Although within-family selection and gynogenesis are helpful to fix good traits in selection programmes, they could result in inbreeding depression if farmers do not pay close attention to the selection of brood fish. At present, inbreeding depression, i.e. retarded growth, short body and orange colour of Jian carp, has been observed in some places. The inbreeding depression was due to a lack of careful selection of brood fish which accelerated the homozygosity of loci controlling the desired traits. It is highly recommended that smallholder farmers should select brood fish each generation when they produce seeds for aquaculture. Alternatively, they may purchase seeds from authorized hatcheries that have well-managed breeding programmes in order to get high-quality seeds.

Training on proper selection of brood fish by smallholder farmers and larger producers has been undertaken by the Freshwater Fisheries Research Center of the Chinese Academy of Fishery Sciences. The training consists of lectures and fieldwork. On average, four to five batches of such training were conducted every year. Each course lasted three days and some 50 farmers and technicians participated. Until now, more than 5 000 farmers have received training on artificial propagation of Jian carp.



Fermented fish
(the bonga,
Ethmalosa
fimbriata) drying
on racks in Tanji,
The Gambia
©FAO/ Alhaji M. Jallow

CHAPTER 4.5

SMALL-SCALE FISH FERMENTATION AND PROCESSING IN WEST AFRICA

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SUMMARY

Small-scale fish¹ processing, including fermentation, in West Africa contributes to solving problems of food insecurity that impede national development and threaten peace in the region. Due to limitations in infrastructure and the prevailing poor technologies, rural areas in West African countries have been slow in keeping abreast of global developments towards industrialization. The lack of standardization of the processing methods and poor hygiene during processing, with their detrimental effects on the quality and the safety of the end products, are the major problems to overcome to ensure the promotion of fermented fish products supply chains in West Africa. Quality, safety and acceptability of traditional fermented fish may be significantly improved through the choice of raw material, good handling practices and the use of starter cultures selected on the basis of multifunctional considerations. Enriched by inputs from genetics and/or genomics research, biotechnology is a major force for development in fish fermentation in West African countries. There is a need for new areas of research focused on the standardization of traditional fermentation techniques and packaging of end products to meet the expectations of modern consumers, enhance value-added products and increase the market share of traditional fermented fish products.

INTRODUCTION

Fermentation is one of the oldest known uses of biotechnology. Fermented fish can be described as any fishery product that has undergone degradative changes through enzymatic or microbiological activities either in the presence or absence of salt (Zakhia and Cuq, 1993). Fermentation is a process by which beneficial bacteria are encouraged to grow. These bacteria increase the acidity of the fish and therefore prevent the growth of spoilage and food-poisoning bacteria. Additionally, salt is used to prevent the action of spoilage bacteria and allows the fish enzymes and the beneficial acid-producing bacteria to soften (break down) the flesh. Fermentation processes are believed to have been developed over the years by women to preserve food for times of scarcity, impart desirable flavour to foods and reduce toxicity (Rolle and Satin, 2002). Today, fermentation is still widely practised as a household or village-level technology in many countries, but comparatively very few operations are carried out at an industrial level (Holzapfel, 2002).

1 “Fish” is used here in the broad sense to include fin fish and other aquatic animals e.g. molluscs.

Fish has been an important part of the diet of West Africans. It is estimated that 15 to 20 percent of all animal proteins come from aquatic sources (FAO, 2012). There is a strong preference for fresh fish. However, cured fish products such as smoked, salted, sun-dried and fermented fish are also popular.

In countries such as Chad, Côte d'Ivoire, Gambia, Ghana, Mali, Nigeria, Sierra Leone, Sudan and Uganda, fermented fish products have been relatively popular (Watts, 1965; Eyo, 1993; Zakhia and Cuq, 1993). For example, fermented fish are reported to have a ready market in the Lake Chad region of northern Nigeria (Azeza, 1986) and in the Delta State of Nigeria with preference for fermented *Heterotis niloticus* (Nwabueze and Nwabueze, 2010).

APPLICATION OF FERMENTATION IN SMALL-SCALE PROCESSING

In West Africa, hot smoking is the main method of fish processing. However, curing by salting, fermentation coupled with drying constitutes the second most important method of preserving fish after smoking. The processes of fermentation and drying preserve and add value to the fish, giving a product which has a characteristic odour and aroma. More income is derived by fish processors from fish fermentation than from smoking or drying (FAO, 1992). This, however, may depend on a wide range of factors such as the species, geographic location, seasonality and physiological state (gravid or not) of the fish, the market structure and the target consumer's purchasing power.

The quantity of fish that is processed into fermented products in any particular country is influenced by the food habits of the people and market demand. Table 1 shows the total domestic annual fish supply, the quantities consumed as fresh or frozen, and the quantities processed into smoked and fermented products by West African countries.

Côte d'Ivoire: About 35 percent of the total annual production of fish is consumed fresh while 50 percent is smoked (FAO, 1992). Only about 10 percent of the total fish supply is eaten as salted, dried and fermented products, mainly as a condiment. However, small quantities are exported to Burkina Faso. The remaining 5 percent may be eaten as fried or charcoal grilled fish, especially for outdoor facilities (restaurants, chop bars and street foods)

The Gambia: About 50 percent of the annual fish production in the Gambia is processed into salted, partially fermented and dried products mainly for export to Côte d'Ivoire, Ghana and Mali. The price of the product on the Gambian local market is high compared to other cured fishery products.

Ghana: About 15 percent of the total annual fish production is fully or partially fermented into either dried or salted dried products for the local market. The product is used as food fish and as condiment in local dishes. Salted and fermented dried fishery products are imported from the Gambia, Norway and Senegal to meet shortfalls in local supply.

Mali: Of the total annual fish production, as much as 75 percent is processed by smoking, wood grilling and dried fermentation. The rate of processing is high in Central Niger Delta where 80-90 percent of fish is processed, but lower in other areas where fresh fish is preferred. Fresh fish consumption in Mali is between 10 and 25 percent compared with 60 percent for smoked fish, which constitutes the largest share of the domestic fish supply.

Senegal: About 10 percent of the annual domestic supply is processed into various dried and fermented products. A large proportion is distributed locally, but significant quantities, representing 2-5 percent of the total volume of fish exports, go to countries such as Burkina Faso, Congo, Ghana, Mali, Togo and the Democratic Republic of Congo.

Nigeria: About 7.4 percent of the annual domestic supply is smoked (FDF, 2010) while the majority is consumed fresh. Fermented fish are mostly imported. Nigeria imports about 60 percent of Chad's annual production of partially fermented, sun-dried fish.

Table 1: Total domestic annual fish supply, the quantity consumed as fresh or frozen, and the quantities processed into smoked and fermented fish products by West African countries (in tonnes).

COUNTRY	ESTIMATED ANNUAL DOMESTIC FISH SUPPLY	QUANTITY CONSUMED AS FRESH OR FROZEN FISH	QUANTITY PROCESSED AS SMOKED FISH	QUANTITY PROCESSED AS FERMENTED FISH
Côte d'Ivoire [#]	75 000	26 250	37 500	7 500
The Gambia [#]	46 000	23 000	Not reported	23 000
Ghana [#]	350 000	54 000	243 500 ^{**}	52 500
Mali [#]	100 000	20 000	60 000	15 000
Senegal [#]	420 000	42 000	75 000 ^{**}	42 000
Nigeria [*]	820 000	754 400	60 680	Not reported

[#]FAO (1992); FAO (2010)

^{*}FDF (2010)

^{**} Estimated

Adapted from FAO (1992) and updated from FAO (2010).

JUSTIFICATION OF FERMENTATION TECHNOLOGY, IMPORTANCE FOR FOOD SECURITY AND THE LOCAL ECONOMY

Fermentation prolongs the shelf-life of fish in addition to improving its nutritional value. Small-scale fermentation technologies contribute substantially to food security and nutrition, particularly in regions that are vulnerable to food shortages (FAO, 1998). Fermentation also improves fish food quality through greater digestibility to increase essential amino acids, vitamins and protein. In addition, fermentation technology provides direct employment for processors and supports others who provide indirect services to the industry in the areas of packaging, distribution, marketing etc.

Fermented fish are generally appreciated for their pleasant flavour, aroma, texture and improved cooking and processing properties. It can even have beneficial health effects when the fermenting micro-organisms possess probiotic activity. Micro-organisms, by virtue of their metabolic activities, contribute to the development of characteristic properties such as taste, aroma, visual appearance, texture and shelf-life. Enzymes indigenous to the raw materials may play a role in enhancing these characteristics (Hammes, 1990).

PROCESS FLOW IN FERMENTED FISH PRODUCTS, SPECIES AND END PRODUCT USAGE

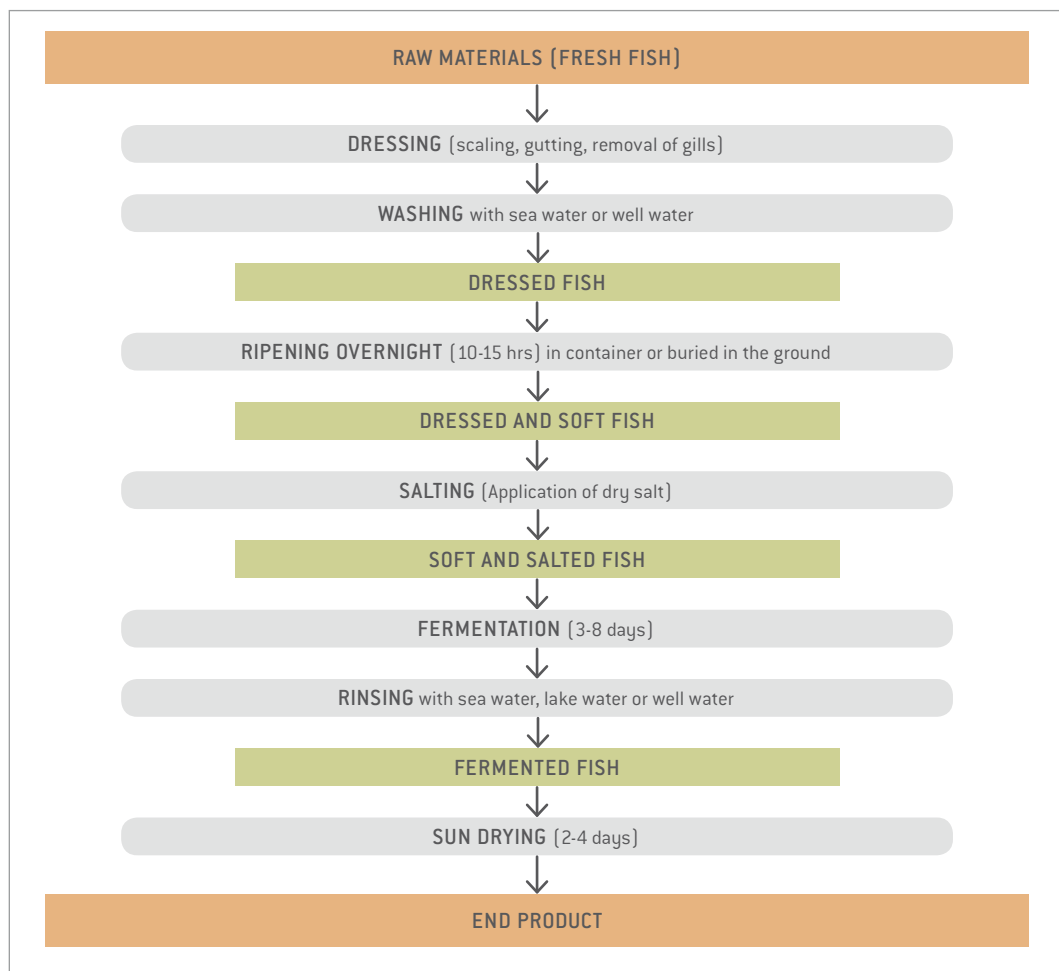
Three basic methods were identified for fish fermentation in Africa: fermentation with salting and drying, fermentation with drying without salting and fermentation with salting but without drying (FAO, 1992; Dirar, 1993 and Anihouvi *et al.*, 2005). The first method is predominantly employed for traditional fish fermentation in West Africa (Figure 1). Anihouvi *et al.* (2012) summarized the major fermented fish products in West Africa, which we shall now consider.

Lanhoun processing: The first step consists of the scaling, gutting and washing of fresh fish. This is followed by 10-15 hours of ripening, during which the fish is left in a bowl without water (Figure 1). The ripening influences the texture and aroma of the end product. After ripening, the fish is salted before fermentation and the salted fish arranged in containers (baskets, cans, baskets lined with cement, concrete vats and earthenware jars), wrapped with jute sacks or cloth, or buried in a 2-m deep hole and allowed to ferment for 3 to 8 days, depending on the local conditions and the kind of product desired (Anihouvi *et al.*, 2005; Kindossi *et al.*, 2012).

The species of fish include; Cassava croaker (*Pseudotolithus senegalensis*), Lesser African threadfin (*Galeoides decadactylus*), Atlantic bumper (*Chloroscombrus chrysurus*), Spanish mackerel (*Scomberomorus tritor*) and Crevalle jack (*Caranx hyppos*).

Momone processing: The processing of Momone is similar to Lanhouin (Figure 1). It is usually carried out to salvage large quantities of fish that would otherwise have been discarded due to poor quality. Momone processing involves the use of whole fish cut into smaller pieces or split dorsally. Dressed fish is washed and either left overnight before salting (15-40 percent by fish weight) or salted immediately after washing and allowed to ferment for 3 to 8 days, followed by drying on raised platforms for 1 to 3 days (FAO, 1992; Abbey *et al.*, 1994).

Figure 1. Flow diagram for traditional processing of fermented fish products in West Africa



Source: adapted from Anihouvi *et al.*, 2012

Guedj processing: Guedj is a Senegalese and Gambian traditional fermented fish product, used as a flavouring agent and very much appreciated by the local populations because of its exceptional flavour and taste (Diop, *et al.*, 2010). Dressed fish are salted and allowed to ferment for about 2 to 3 days, followed by the drying on raised platforms for about 3 to 5 days (Figure 1). For marketing purposes, the women also smear the fish with an oil-based substance that gives it a reddish colour, conforming to the preferences of Senegalese consumers. Different fish species are used for the preparation of guedj, including mackerel, seabream, threadfin, croaker, mullet, catfish, meagre, herrings, skate, rays and shark, which are caught with passive nets, beach seines and lines.

Yeet: Yeet is mainly a Senegalese fermented product prepared from cymbium, a sea snail (gastropod). The flesh is removed from the shell, split into two to four parts and fermented by burying the product for three to four days in sand and covered with a canvas to prevent exposure to air. In response to better quality product, fermentation takes place in plastic containers filled with seawater and dried on raised platforms for a month to give a reddish coloured product, as demanded by Asian consumers (ICSF, 2002).

Other West African fermented fish products: There are many other fermented fish products which have received little scientific investigation. These products are: Malian Djege/Djadan and Ivorian Gyagawere/Adjonfa. Dressed and headed fish are salted and then put into water in an earthenware pot or oil drum and allowed to ferment for 12 hours (Figure 1). Fish species such as *Tilapia*, *Clarias* spp., *Alestes* spp., *Schilbe* spp. and *Hydrocynus* spp. are commonly used to process Djege and Djadan. Some major fermented fish products from West Africa and micro-organisms involved in their production are presented in Table 2.

SAFETY, QUALITY AND MARKETING CONSIDERATIONS IN SMALL-SCALE FISH FERMENTATION PROCESSING

Traditional fish fermentation processes are typically uncontrolled and dependent on micro-organisms from the environment or the fermentation substrate to initiate it, and as such can result in products of variable quality. Fermentation is generally considered to preserve or keep the hygienic quality and safety of foods, but if it fails, spoilage may result and pathogens survive, thereby creating unexpected health risks in food products which would otherwise be considered safe (Holzapfel, 2002). There is a widespread perception among fish operators that spoiled fish can occur through smoking, drying or fermentation. Besides, there is confusion in most instances between fermentation and spoilage. All these do not provide room for good

quality raw material, and therefore lead to poor quality end product. At all times, the theory of starting with good quality raw materials resulting in good quality end products should always hold. Quality, safety and acceptability of traditional fermented fish may be significantly improved through the use of starter cultures selected on the basis of multifunctional considerations.

Poor processing of traditional fermented fish products constitute health hazards to consumers. Such hazards can arise through the processing technique, environment, the waste disposal system, the unhygienic nature of processing materials and improper packaging of the end products. The use of water from lagoons, rivers, lakes or the sea can introduce products to possible chemical and microbial contamination (Dirar, 1993; Anihouvi *et al.*, 2005; Anihouvi *et al.*, 2012). Drying on the ground leads to contamination with sand and micro-organisms as well as blowflies and other insects, which lead to the illegal use of insecticides (FAO, 1992; Anihouvi *et al.*, 2005; Abbey *et al.*, 1994). Inappropriate technologies and lack of standards in the fermented fish products industry could be potential vehicles for transmission of food-borne diseases. There have been confirmed high levels of biogenic amines, mainly histamine, in various fermented fish products (Anihouvi *et al.*, 2005; Abbey *et al.*, 1994; Wootton *et al.*, 1989).

According to Yin *et al.* (2002) the use of starter cultures of lactic acid bacteria to ferment minced mackerel could suppress the growth of spoilage bacteria and pathogens, and substantially inhibit the development of volatile basic nitrogen. Using starter cultures for fish fermentation could also reduce the fermentation time, enhance the inhibition or elimination of food-borne pathogens, and improve the shelf-life and sensory quality of products in terms of taste, aroma, appearance and texture.

In the West African sub-region, the flow of fermented dried fish is mainly from the Gambia, Mali and Senegal to Burkina Faso, Côte d'Ivoire, Ghana, Nigeria and Togo. Senegal exported fermented fish to various West African countries. Small quantities of salted/fermented dried fishery products were exported from the Gambia and Senegal to France where they were patronized by the resident West African communities (FAO, 1992). The bulk of fermented fish produced in Côte d'Ivoire, Ghana and Mali were marketed locally. The product lends itself to easy packaging, transportation, distribution and marketing without chilling or any other expensive method of storage.

Table 2. Some major fermented fish products from West Africa and the micro-organisms involved in their production.

TYPES OF FISH	PRODUCTS LOCAL NAME	COUNTRY	FERMENTATION DURATION	MICRO-ORGANISMS INVOLVED
Catfish, croaker, meagre, shark, mullet, skate, rays, triggerfish, horse mackerel, octopus, tuna, sole, Spanish mackerel, seabream, herring	Gyagawere, adjuevan	Côte d'Ivoire	6 hours to 3 days with salting	Lactic acid bacteria <i>Leuconostoc lactis</i> <i>Lactobacillus fermentum</i> <i>Pediococcus</i> sp, <i>streptococcus</i> sp
Cassava croaker/Cassava fish, kingfish	Lanhouin	Benin	3-8 days with salting	<i>B. subtilis</i> , <i>B. licheniformis</i> , <i>B. megaterium</i> , <i>B. cereux</i> , <i>B. Mycoides</i> , <i>Micrococcus luteus</i> , <i>Staphylococcus lentus</i> , <i>Staphylococcus xylosus</i> , <i>Streptococcus</i> ; <i>Corynebacterium spp.</i> ,
Catfish, barracuda, seabream, threadfin, croaker, grouper, bonito, mackerel, herrings, squid, octopus, bumper, snapper, ribbon fish	Momone	Ghana	Overnight to 3 days with salting	<i>B. subtilis</i> , <i>B. licheniformis</i> , <i>B. megaterium</i> , <i>B. cereux</i> , <i>B. mycoides</i> , <i>Micrococcus luteus</i> , <i>Staphylococcus spp</i> , <i>Lactobacillus</i> , <i>Pseudomonas</i> , <i>Pediococcus</i> , <i>Klebsiella</i> , <i>Debaryomyces</i> , <i>Hansenula</i> and <i>Aspergillus</i>
Carp, threadfish	Djegue, jalan	Mali	Overnight, no salting	unknown
Alestes Nile perch, parch	Aku	Ndokwa-East in Southern Nigeria	Overnight, with little salt	unknown
Mackerel, seabream, threadfin, croaker, mullet, catfish, meagre, herrings, skate, rays, shark	Guedj, tambadiang, yet	Gambia, Senegal	Overnight to 2 days with salting	<i>Proteus spp.</i> , <i>Shewanella</i> , <i>putrefaciens</i> , <i>Bacillus spp.</i>

adapted from Anihouvi et al., 2012

PERSPECTIVES AND VIABILITY OF BIOTECHNOLOGY IN SMALL-SCALE FISH FERMENTATION IN WEST AFRICA

The greatest drawback in the development of fermented fish products in West Africa is that many are produced under primitive conditions, resulting in low productivity, poor quality and short shelf-life. With improved technologies, it should be possible to be innovative in adding value, such as increased shelf-life, flavour and appealing packaging and labelling, to fish products using fermentation and indigenous knowledge systems.

There are many opportunities for biotechnological innovation in the microbiology of fermented fish such as isolation, characterization and preservation as a germplasm and collection of all the microorganisms involved; understanding the metabolic role of each of the strains involved, and their full potential in other fields of biotechnology. The powerful technique of monoclonal antibodies for the characterization of different strains of the same species can be of great help in this area.

Fermentation biotechnology has also given rise to great innovations in engineering designs of bioreactors. Most of these designs deal with liquid reaction media, but traditional fermented fish products in West Africa are produced through solid-substrate fermentation. Bioreactors to simulate such process are needed for the modernization of the traditional fermented fish production.

CONCLUSION

Small-scale fish fermentation is desirable in terms of food preservation, quality and nutrition. However, the safety, nutritional and flavour profile of the products needs to meet the expectations of consumers. The lack of standardization of the processing methods and hygiene impairs the quality and safety of the products. The packaging of the end products needs to be improved while the development of local food industries through the improvement of traditional fermentation technologies is one of the ways to enhance value-added products and the market share of traditional products. In this regard, the culture, culinary traditions and the current level of development of the processing methods in each country should be taken into account.



from left to right

Dried fermented fish arranged on elevated racks in the Gambia
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In Ghana retail trading of fermented fish is exclusively a female activity
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CHAPTER 5

AGRICULTURAL BIOTECHNOLOGY CASE STUDIES: CHALLENGES, ACHIEVEMENTS AND LESSONS LEARNED

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EXECUTIVE SUMMARY

This chapter summarizes the background, challenges, results and lessons learned from the 19 case studies described in the preceding chapters of this book. The coverage in terms of regions, production systems, species and underlying socio-economic conditions was extensive, as were the technical challenges faced by the smallholder farming communities at the centre of the studies, as they and their national and/or international partners sought to improve livelihoods by using biotechnology to boost productivity and market penetration and better manage natural resources.

Most of the case studies involved application of a single biotechnology in a single species. They spanned the crop (seven case studies), livestock (seven) and aquaculture/fisheries (five) sectors and, apart from one dedicated to West Africa, focused on a specific initiative within a single country. Four case studies were from India, two from China and one each from Argentina, Bangladesh, Brazil, Cameroon, Colombia, Cuba, Ghana, Nigeria, South Africa, Sri Lanka, Tanzania and Thailand. A wide range of biotechnologies was used in the case studies, including some of the oldest or “traditional” methods such as fermentation, as well as several now at the forefront of “modern” science involving highly sophisticated DNA and genetic analyses, although not including genetic modification.

In terms of results, the outputs were many, varied and valuable in terms of the scientific and technical knowledge and products that were generated and the strengthening of human and infrastructural capacities that was achieved. Collectively, these also had great potential for improving on-farm productivity, market access and livelihoods. While evidence of significant outcomes (i.e. widespread adoption or use of the products by farmers and supporting partners such as extension agents and policy-makers) was not convincing in all cases, some technologies, particularly in relation to seed crops and fish, have been adopted “big time”. In the case of livestock and vegetatively propagated crops, the rate of adoption was less spectacular but nonetheless certainly meaningful to the farming communities concerned. Plausible evidence that the biotechnologies described had economic, health or environmental impacts on the lives of producers and consumers was lacking in most cases, but clear-cut for a small number of studies.

Ten general and interrelated lessons can be drawn from the case studies to guide future agricultural research for development (AR4D) investments in biotechnologies. These include: the absolute necessity for government policies and backing from donors and intergovernmental

agencies, and of partnerships both within and outside the public sector and with the farmers themselves in the planning and implementation of projects and programmes while bearing in mind also the need to retain flexibility in order to respond appropriately to evolving circumstances; and the recognition that while investments in science and technology are critical, the successful use of biotechnologies also requires their appropriate integration with other sources of science-based and traditional knowledge.

Other lessons learned from the case studies are that AR4D involving biotechnologies need not be constrained by questions involving access to, or use of, genetic resources or issues of intellectual property rights, and that products developed through biotechnologies do not need to conform to specific biosafety and food safety regulations or standards. Finally, the studies indicated that it is essential to strengthen the planning, monitoring and evaluation of biotechnologies for agricultural development. Institutional arrangements and skills in these areas are currently weak or non-existent, and should therefore be strengthened to enable governments and donors to properly evaluate and justify the financial and other investments they allocate to agricultural biotechnologies.

INTRODUCTION

All farmers are unique. The biological, physical and economic resources that they use and the activities they pursue to produce food and other agricultural products are diverse and complex. The case studies in this book certainly illustrate the immense diversity both of the challenges and of the opportunities facing smallholder farmers in developing regions. However, on closer analysis, the fundamental problems they share – and the approaches being employed to tackle them – are often similar and cut across institutional, political and national boundaries.

For a number of reasons, this chapter does not judge which of the 19 case studies resulted in the greatest hunger reduction or economic growth (or the potential for doing so in the years ahead), although several appear to have contributed greatly. The first reason is that the biotechnology interventions described set out to change only one of the many resources (e.g. usually a crop or animal species) available within the broader farming systems in which the target smallholder beneficiaries found themselves. Most likely, therefore, their livelihoods were supported by a range of interdependent activities besides producing crops, livestock and/or fish – including through the off-farm rural economy. Their livelihoods were also inevitably influenced greatly by the wider external environment – input and output markets, institutions, policies etc.

Secondly, because of the general paucity of information provided concerning farming systems, livelihoods, coping mechanisms, household food security and the like, reaching conclusions concerning the “people impacts” of (most of) the biotechnologies described here is not possible. Nor is it possible to evaluate whether, for example, the investment priorities as described in the cases studies were really the most appropriate for helping the intended beneficiaries realize their ambitions. Clearly, however, the decisions to proceed along the lines described in the studies were based on agreements within the national agricultural research systems (NARS) themselves and supported by national and/or international funding agencies and other bodies, and need not be discussed further.

The purpose of this chapter, then, is to summarize the interventions in the light of three well-established household, national and international routes for escaping or avoiding hunger, malnutrition and poverty by promoting technology change within smallholder agricultural systems, namely: sustained productivity growth of existing production patterns; diversification of activities into higher-value horticultural, livestock and aquaculture products to exploit new or existing market opportunities and thereby increase farm incomes; and enhanced stewardship of natural resources to secure the long-term sustainability of farming enterprises.

Elements covered in this chapter include: the challenges or needs addressed; the choices made in terms of the species employed and biotechnological intervention(s) made; the results obtained in terms of outputs, outcomes and impacts; and the lessons learned. Included here are suggestions for improving the rigour with which the performance of agricultural research for development (AR4D) projects involving biotechnologies may be planned and monitored and their prospects for both funding and success improved.

THE MANY CHALLENGES

Smallholders have many hurdles to overcome when trying to increase productivity, diversify the portfolio of products for marketing and home consumption, and improve their management of the natural resources available to them for sustaining their livelihoods. Many arise from the policy and institutional environments in which they have to operate, and some of these are illustrated later. Others relate to the biophysical characteristics – the natural resources and climate – of the farming systems themselves. Often, farmers, particularly smallholders, have limited or no control over these attributes. Nevertheless, as shown by undeniable past successes, science and technology coupled with improved human capital have been powerful drivers of positive

change in the performance and evolution of smallholder systems. The case studies described in this book all set out – through biotechnologies – to reduce the impediments to productivity and provide new or better products in challenging biophysical environments.

Although confined to just 19 studies, the studies illustrate all too well the great variety of technical constraints that needed to be tackled. For example, in the crop sector, efforts were directed at six species (banana, cassava, pearl millet, plantain, rice and sweet potato), all of which are widely grown as subsistence crops by smallholder farmers and are considered so critical to food security that they are covered under the International Treaty on Plant Genetic Resources for Food and Agriculture (www.planttreaty.org/content/article-xiv). More than 20 challenges were described, including: insufficient availability, high costs and poor quality of planting material due to viruses, bacteria and fungi; susceptibility to diseases, drought, flooding and storms; and slow growth and late fruiting. All of these, alone or in combination, reduced yields.

Concerning livestock, the emphasis was on dairy cattle in peri-urban settings, and on goats and sheep which are particularly important for the livelihoods of smallholders living in some of the harshest environments. Here, the main challenges included: identifying and breeding more productive animals and tackling the many causes of low reproductive efficiency to improve milk, meat and wool off-takes, quality and marketing; conserving an endangered sheep breed; diagnosing, controlling or eradicating transboundary diseases, like peste des petits ruminants (PPR) – also known as goat plague – and pests like the tsetse fly, arguably the biggest constraint to livestock and wider agricultural development in sub-Saharan Africa; and converting pig effluent into energy, biofertilizer and biochar for improving soil health and increasing milk, crop and wood production on land provided for resettlement by smallholders.

A similar picture emerged from the case studies dealing with the fishery sector, illustrated by efforts to reduce disease loads, improve yields and counter the environmental footprint and food safety concerns arising from shrimp production; to produce catfish which were more disease-resistant, faster-growing and produced better quality meat; to improve the production and quality of carp and to reduce losses due to spoilage between harvest and consumption of caught fish.

Overall, therefore, the coverage of these case studies demonstrated a clear smallholder focus in terms of species, constraints, needs, opportunities and intended benefits.

THE BIOTECHNOLOGY RESPONSES

BIOTECHNOLOGIES USED IN THE CASE STUDIES

In 2009, as part of the build-up to the FAO international technical conference on Agricultural Biotechnologies in Developing Countries (ABDC-10), FAO organized an e-mail conference which provides a good overview of the main kinds of agricultural biotechnologies being used in developing countries. This information, and the sector-specific technical documents prepared for ABDC-10, are available in FAO (2011a) and need not be repeated here.

The biotechnologies most frequently represented in the case studies of this publication used one or a combination of two DNA-based detection methods, namely restriction fragment length analysis and the polymerase chain reaction (PCR), including various more recent innovations thereof, e.g. loop-mediated isothermal amplification (LAMP). These were employed for a wide variety of purposes and across all sectors.

In the crop arena, they were used for molecular markers, such as restriction fragment length polymorphisms (RFLPs) and simple sequence repeats (SSRs) or microsatellites, to detect and validate quantitative trait loci (QTLs) for particular traits. These were then transferred into elite parental lines using marker-assisted backcrossing (MABC) to produce downy mildew-resistant and drought-tolerant pearl millet in India, rice tolerant to submergence in India and cassava mosaic disease (CMD) resistant cassava in Nigeria. In the livestock sector, DNA markers (PCR combined with RFLP) were used in India to detect FecB and introgress this prolific gene into the more productive Deccani breed through breeding and backcrossing with the smaller Garole breed which carried the gene, and in South Africa to determine the genetic diversity within, and conserve, also with the help of embryo cryopreservation, the Namaqua Afrikaner sheep breed, which is threatened with extinction. A further application was to quickly diagnose PPR in goats using LAMP, thereby enabling veterinary authorities in Cameroon to stamp out local outbreaks and stop the spread of this fatal disease to other farms. In aquaculture also, the value of PCR-based tests was amply demonstrated in India for pathogen (mainly virus) screening and for the control of disease outbreaks in shrimp farming.

Featuring prominently among the biotechnologies used in the crop sector case studies were tissue culture and micropropagation. When combined with molecular diagnostics (and in one case specific antibodies in an enzyme-linked immunosorbent assay [ELISA]), they generated

large amounts of planting material of preferred varieties free of diseases such as frog skin disease, banana bunchy top virus and “Black Sigatoka”, for distribution to farmers. Four case studies within the crop arena employed this approach (Colombia, Ghana, Nigeria and Sri Lanka), while the study in Cuba described the production and evaluation of plantain plants in tissue culture from somatic embryos.

Reproductive biotechnologies, most notably artificial insemination (AI) using cryopreserved semen, to achieve both pregnancy and genetic improvement of cattle, goats and catfish were the focus of the three case studies from Bangladesh, Argentina and Thailand respectively, and to some extent also of the efforts to improve Deccani sheep in India. A study from China also illustrated a further avenue for achieving genetic improvement, namely by producing lines of carp from the eggs of females selected for good traits through traditional breeding, which had been inseminated with inactivated sperm – a process called gynogenesis.

Other biotechnologies were less prominent but were deployed to tackle no less important issues. For example, ionizing radiation was used to sterilize male tsetse flies (known as the sterile insect technique [SIT]) for release on Unguja Island, Zanzibar, and to induce mutations and create shorter and earlier-maturing banana plants in Sri Lanka.

Two case studies from China and Brazil employed other biotechnological approaches, inspired by the need to respond to the environmental concerns arising from intensive agricultural practices. In China, bacteria (probiotics) were identified and employed as alternatives to antibiotics for improving water quality while reducing pathogenic bacteria in shrimp farming. In Brazil, the fermentation of pig effluent was used to produce biogas and biofertilizer. This same project also subjected the effluent to pyrolysis to produce biochar – a solid fertilizer to improve soil health and crop production.

Finally, the case study from West Africa described the use of fermentation – possibly the oldest biotechnology known – to preserve fish and thereby cut food wastage while at the same time improving palatability, nutritional value and marketability. Noteworthy here is the almost complete reliance on largely uncontrolled practices at household and village levels that rely on a combination of micro-organisms from the environment and traditional knowledge and experience to process raw produce into acceptable food products.

JUSTIFICATION FOR USING BIOTECHNOLOGIES

The achievements described in these case studies are summarized in the next section, but by way of “setting the scene” it is worth considering why the biotechnologies were employed in the first place. What made these propositions attractive to the people (scientists) who researched their development and encouraged their adoption by farmers? The answers provided are certainly convincing.

DNA detection methods, for instance, offered levels of specificity and sensitivity that could not be otherwise achieved for characterizing agricultural genetic resources and identifying organisms that can enhance or stunt productivity. They could also do this faster, thereby cutting breeding time and effort, and they could diagnose diseases that spread rapidly within and between fields, herds and ponds much quicker than other ways, and thereby both protect and enhance smallholder assets.

Tissue culture and micropropagation seek to ensure that farmers have sufficient, “clean” and uniform planting materials of vegetatively propagated crops at the time they are needed. Theoretically, millions of such plants could be produced in a very short time, whereas the alternative of producing these from stem or root cuttings is slower, less reliable and vulnerable to the dissemination of pests and diseases.

Artificial insemination together with semen cryopreservation relieve smallholders of the burden of keeping live males and provide a means for farmers both to ensure that their animals are bred at the correct time in their reproductive cycles and to improve their livestock using males that have desirable characteristics. Additionally, genetic material can be stored which can improve both the planning and timeliness of inseminations in outlying areas. The techniques for controlling reproduction and selecting faster-growing and more disease-resistant aquatic species offer similar advantages.

Other benefits offered by the biotechnologies described included: the prospect of getting completely rid of disease-causing pests without having continuously to use pesticides that also kill beneficial insects; using bacteria instead of antibiotics to promote animal, human and environmental health; employing biofertilizers instead of chemicals to improve productivity while lowering environmental, human health and food safety risks; and, in the absence of “cutting-edge” science and modern equipment, to produce fishery products with a longer “shelf life”, thereby reducing wastage and improving access to products with high nutritional value.

But what do the present case studies reveal about the usefulness of the biotechnologies to smallholder farmers, the beneficiaries whose welfare is the central concern of this publication? What about the realities of developing and releasing biotechnologies into fields and ponds and pastures?

THE RESULTS: OUTPUTS, OUTCOMES AND IMPACTS

Expectations are high that biotechnology will be a key ingredient within the mix of approaches taken by countries for increasing agricultural productivity and contributing to wider food security objectives such as improved nutrition and natural resource management. Volumes have been written about how to assess the consequences of all kinds of research effort, and both the methodologies and the caveats surrounding their usefulness are many: these are not described here. For readers interested in mapping the performance of agricultural research, the website of the Consultative Group on International Agricultural Research [CGIAR] Standing Panel on Impact Assessment [SPIA] is the best source of information [<http://impact.cgiar.org/>].

However, at its heart, assessing whether programmes and projects, or technologies used within them, actually make a difference revolves around collecting, analysing and linking in a plausible manner information about both the inputs made and the outputs, outcomes and impacts delivered. Critically, it also depends on who is expected to benefit – the smallholder(s) themselves and/or the wider rural and even urban population – and in what way, whether through higher yields, saved labour, improved nutrition, greater economic returns etc.? Untangling all of this is a formidable challenge, further complicated by the often significant time lags between research outputs and their transmission to end-users and beneficiaries, and the difficulties in establishing appropriate counterfactuals to improve the rigour of attribution [what if, for example, the biotechnology had not been employed?] Nevertheless, research institutions have a responsibility to make sure that the research they undertake is geared to the development needs of their countries, and to do so they must examine its performance. Put simply, they need to ask: what worked and did not work, and why. The result is a learning process through which focus can be sharpened and performance improved, and national planners and investors can be reassured that the paths proposed from research to development are as sound as possible, in view of the risks inherent in both conducting research and having the results adopted.

Below is an attempt to help the reader draw his/her own conclusions about what has been achieved within these case studies – technically, agronomically and from a socio-economic perspective.

OUTPUTS

The case studies presented in this book describe very many tangible outputs from the research conducted: new or innovative techniques; better crop and animal genotypes, products and processes; new laboratory and plant propagation facilities, including some (in Colombia, Ghana and Sri Lanka) run by farmer and community groups; smarter ways of doing things such as marker-assisted, instead of conventional, backcross transfer of useful traits; trained scientists, technicians, farmers, extension officers, labourers and even schoolchildren.

These outputs, if adopted widely and managed appropriately, would surely have great potential for improving livelihoods. The disease-free cassava, banana, plantain and sweet potato plants, the disease-resistant and drought-tolerant pearl millet, the molecular markers and DNA diagnostics for localizing important agronomic characters and fighting diseases, the quality semen stored in the deep-freezer, the fish that grow faster and the sheep that produce more lambs in experimental stations are just some of the other outputs. All of them are great successes in terms of the traditional “deliverables” expected from research enterprises, all are clear demonstrations of grand scientific and technical endeavour, knowledge generation, structural and human capacity-building. No doubt, these successful R&D enterprises also generated other important but largely hidden results in the form of scientific publications, protocols, guidelines, presentations and briefs to scientists, extension agents, farmer groups and policy-makers, which will have led in turn to stronger or wider partnerships, new grants, etc.

OUTCOMES

Outcomes are defined here as the “adoption or use of research using biotechnology by farmers, extension agents, hatcheries, commercial companies, policy-makers and policy-implementing institutions such as ministries and regulatory bodies”.

By this definition, the case studies are not uniformly persuasive in terms of delivering outcomes to scale or significance. Notwithstanding the caveats mentioned earlier, this analysis suggests that while progress was made by all in moving from research results to uptake by farmers, and by policy-making and implementing agencies, deficiencies in the amount and/or quality of information available make objective judgement difficult, and comparative assessment

impossible. Nevertheless, some technologies were taken up “big time” or at least to an extent that was meaningful in the areas or systems of the countries concerned. For example:

- The HHB 67 Improved pearl millet hybrid developed by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and partners through MABC was released by the Indian government for cultivation in 2005 as a more downy mildew-resistant and higher-yielding replacement for HHB 67. Adoption by both the seed industry and resource-poor farmers in northwest India was massive. By 2011, its cultivation had spread to almost 900 000 ha of land in northern India. Its higher yield promoted crop diversification into cash crops such as sesame and food legumes. Other attributes include early maturity, thereby escaping early-season drought. The short duration of the new hybrid also facilitated the growing of winter season rotational crops such as mustard and chickpea, thereby doubling cropping intensity. For more details, see case study (CS) 2.2 (i.e. Chapter 2.2 of this book).
- The Swarna-Sub1 semi-dwarf rice lines were developed by the International Rice Research Institute (IRRI) and partners using SSR markers to identify a major QTL for submergence tolerance and to transfer it thereafter through MABC. They were released for commercial cultivation in India in 2009 by the Central Rice Research Institute (CRRI) in Odisha and the Narendra Dev University of Agriculture and Technology (NDUAT) in Uttar Pradesh. The state governments of Uttar Pradesh, Bihar, Odisha and West Bengal then initiated large-scale seed production and dissemination. Around 38 000 tonnes of Swarna-Sub1 seed were produced in 2011, reaching over three million farmers and covering over one million ha during the 2012 wet season. These lines are highly tolerant to submergence and lodging and both improve and stabilize yields in villages prone to flooding and areas affected by flash floods. Depending on the severity of flooding, they produce 1-3 tonnes more rice per ha than Swarna, currently the most popular variety grown in the rainfed lowlands of India. There are no yield and grain quality penalties associated with Swarna-Sub1 lines in non-flood years and they provide greater opportunities for adjusting cropping patterns. The Bill and Melinda Gates Foundation supports the project “Stress-Tolerant Rice for Africa and South Asia” (STRASA), which is helping to extend the benefits of Swarna-Sub1 both within and outside India (for more details, see CS 2.7).
- The disease-free or “clean” sweet potato plant materials produced through tissue culture and micropropagation were distributed to 14 500 smallholders in different parts of Ghana. The sweet potato varieties reduced disease pressure and produced yields of 11-15 tonnes per ha. Plantain “seedlings” (2 000) were bought by individuals, non-governmental and religious organizations for field establishment. Banana and plantain hybrid plantlets were also evaluated by 1 000 farmers in Ghana. Reported advantages included 60 percent more, and 30 percent heavier, banana hands and 10 percent thicker plantain plants; better fruit juice was also mentioned (CS 2.3).
- A community-based foundation in Bangladesh now provides production-related veterinary services, including AI, to around 3 000 smallholder dairy cattle farmers. The number of farmers joining the programme has jumped in the last three to four years. Milk production has increased between three- and almost 200-fold in the dairy-producing areas of Satkhira and Sirajgonj and by five times in the Chittagong area. Milk quality has also improved (CS 3.4).
- The best Angora goat breeders in northern Patagonia (Argentina) multiplied genetically superior animals produced using laparoscopic AI. The number of animals under the scheme increased four-

fold within three years and male kids were distributed to smallholders rearing lower quality stock. A certification scheme was introduced by the Ministry of Agriculture to promote marketing of the mohair produced. The volume of certified quality mohair increased from 4 200 kg collected from 19 smallholders in 1998, to 90 000 kg from 830 smallholders in 2006 (CS 3.3).

- The Tanzanian government approved use of the SIT against tsetse flies. Millions of sterile flies were mass-reared in a special facility and released and the tsetse fly was driven to extinction on Unguja Island in Zanzibar. The percentage of small farmers with indigenous cattle increased three-fold and those holding improved breeds increased by a factor of 12, milk production nearly tripled, and ploughing with oxen improved labour and crop productivity. Wildlife management programmes initiated after tsetse eradication increased the numbers of some rare and protected wildlife species. Arising from the success of this project, the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) was established at the African Summit of 2000 under the African Union Commission (CS 3.7).
- Interspecific catfish hybrids produced by AI in Thailand were adopted by at least 1 000 hatcheries and by a far greater number of grow-out farms. They boosted the country's annual production of walking catfish (i.e. mixed species of the genus *Clarias*) from 18 000 tonnes in 1990 to 159 000 in 2004. Among freshwater commodities in Thailand, hybrid catfish production is currently second only to Nile tilapia. Advantages of the hybrid include improved growth rate, survival rate and better quality meat (CS 4.3).
- The Jian carp, developed through a combination of crossing purple red carp with Yuanjiang carp and gynogenesis was distributed to 27 provinces, municipalities and autonomous regions in China following training of more than 5 000 farmers. About 160 000 farms use the fish and it now occupies over 50 percent of the total common carp production in the country. The yield of Jian carp is over 30 percent higher than other varieties of common carp (CS 4.4).
- Several probiotic strains, added directly to water or incorporated into feeds to improve water quality, disease prevention and growth rates, have been approved by the Ministry of Agriculture in China. More than 400 companies now produce probiotics for agriculture in China, developed through research by the private sector alone or by public-private sector partnerships. Annual probiotic use in Chinese aquaculture is currently around 2 000 tonnes, far short of demand which is reported to be 30 000 tonnes, and it is now popular in commercial production of farmed shrimp. A ban imposed by the European Union (EU) because of antibiotic residues in shrimp meat was lifted in 2004. Advantages of probiotic use include much higher growth and survival rates and yields, and better water quality (ammonia levels lowered by 60 percent and nitrate levels by almost 90 percent) (CS 4.1).
- PCR-based shrimp pathogen detection services in India are sold by a number of commercial companies and employed on-site by over 100 registered hatcheries for broodstock and postlarvae screening. They have now reached over 10 000 shrimp farmers in 150 aquaculture societies in five coastal states. Disease prevalence among enterprises run by farmer societies dropped from 80 percent to 20 percent after best management practices, including PCR-based screening, were implemented (CS 4.2).
- Of the approximately one million tonnes of fish captured annually in five West African countries, about 140 000 tonnes are processed by small-scale fermentation – a figure only slightly lower than the amounts consumed as fresh or frozen fish (CS 4.5).

Other case studies describe somewhat more modest levels of uptake or acceptance by farmers. In a number of instances, no information is provided about the number of farmers actually involved, making it impossible to gauge accurately the scale of either early or subsequent uptake. Consequently, while “successful” from the standpoint of moving the technology concerned or the breed out of the laboratory or field station into the care of smallholders, substantive scale-level outcomes remain to be demonstrated. That said, it should be emphasized that, in some cases, the work was underway for less than five years, suggesting that further significant outcomes are possible and even highly likely. In others, the efforts appear to have been ongoing for longer, which suggests the presence of serious constraints to further positive developments.

Examples of case studies falling into the broad category of “work in progress” include four of the studies dealing with tissue culture-based micropropagation of vegetatively propagated plants, although each succeeded in producing plant materials – some with well-defined useful agronomic characters:

- In the case of Cuba, the technology was transferred to six commercial bio-factories which produced 44 000 plantain plants from somatic embryos and had these evaluated positively by local farmers. Bunch weights, hand and finger numbers were greater than in plants produced from corm buds. The material was planted and evaluated on 10 farms managed by individual small-scale farmers or on larger farms managed by farmer cooperatives in four provinces (CS 2.5).
- In Colombia, technology developed by the International Center for Tropical Agriculture (CIAT) and partners for propagation of disease-free cassava was transferred to a rural tissue culture laboratory run by a women’s cooperative for local plant production (CS 2.6).
- In the case of Sri Lanka, *in vitro* micropropagation of banana was transferred from a research laboratory to a tissue culture facility based in a community service centre and over 15 000 plantlets were produced per month. Around 2 500 ha of land were converted from rice to banana cultivation and about 500 farmer families cultivated tissue culture-derived Embul banana cultivars. The plants are shorter, less susceptible to storm damage and produce fruit earlier. Mutants were obtained for earliness and dwarfness (CS 2.1).
- Through collaborative work with CIAT and the International Institute of Tropical Agriculture (IITA), CMD-resistant varieties and landraces were crossed with germplasm from Latin America and, following selection of resistant genotypes based on molecular markers, a few thousand CMD-resistant *in vitro* cultured cassava plantlets were shipped from Latin American to Africa and Asia. These included over 30 000 plantlets, representing over 700 genotypes, that were shipped to Nigeria for use in breeding programmes. As a result of this initiative, two CMD-resistant cultivars, one with a starch content of 27 percent, were released to farmers in Nigeria in 2010 and 2012 (CS 2.4).

Similarly, the case studies involving the *FecB* gene in India, to diagnose PPR in goats in Cameroon, to conserve the Namaqua Afrikaner breed and to convert swine effluent in Brazil might also be defined as “work in progress”. Like some of the crop studies, each of these is in the early stage of implementation but has produced some significant outcomes that include:

- In the Indian state of Maharashtra, rams and ewes of the Deccani breed carrying the *FecB* gene for prolificacy were introduced into 36 smallholder flocks; a number of these are now designated as multiplier flocks providing *FecB*-carrier ewes to other farmers. These sheep produced 27 to 46 percent more saleable lambs than sheep without the gene (CS 3.1).
- Controlling potentially fatal PPR outbreaks in sheep and goats on two farms in Cameroon and preventing spread of the disease to others through the rapid implementation of mass vaccination and animal movement controls (CS 3.6).
- Phenotypic and genetic characterization of three flocks of Namaqua Afrikaner sheep in South Africa, establishing a biobank with frozen embryos and, in 2011, providing surplus young rams and ewes to six farmers (CS 3.2).
- Establishment in Brazil in 2008 of a pilot/demonstration farm unit for 13 selected smallholder farmers with biodigesters, an engine that converts biogas into mechanical or electrical energy for spreading the biodigested swine effluent onto pasture, crop and forest land, and both the machinery and process for producing biochar using the biogas energy and nutrient dense effluent. Farmers were able to double milk production and increase maize and soybean productivity thanks to the soil and nutrient improvements (CS 3.5).

IMPACTS

Here, impacts are defined as “economic, environmental, health and other social benefits and costs to direct adopters of the technology and to those indirectly affected such as input and output marketing agents, labourers and consumers; included are those affected by related policy changes”.

The main point to make here is that impact assessment is about finding out whether, to what extent and how, hopefully for the better, the technology has changed the lives of adopters and wider society. Advanced methods are available for predicting and quantifying the direct and indirect economic or “production effects” of research through *ex ante* and *ex post* assessments using cost-benefit analysis approaches. However, quantifying and integrating environmental, health and other social consequences into impact assessments is both conceptually and methodologically much more difficult even at the level of an individual farm or household, let alone at a village level or on a larger scale. Computable general equilibrium models have been

employed for widening the scope of the assessments and more recent modelling approaches are now available for assessing environmental impacts (Bennett, 2011).

Concerning the linkages between agriculture, nutrition and health, the complexity of this issue and how, among other interventions, agricultural yields and productivity (the present focus of most biotechnology AR4D) can contribute more effectively to better nutrition and health outcomes, are reviewed elsewhere (World Bank, 2007). Also, by embarking recently on a research programme on agriculture for nutrition and health (A4NH) aimed at reshaping agriculture towards these ends, the CGIAR is attempting to fill important gaps in knowledge and methodology. It is already clear, however, that this will require substantial data and considerable integrative skills in economics and in the social and environmental sciences and validation for agricultural development research settings. Agreeing on methodologies appropriate for quantifying the social and environmental consequences of technology adoption is therefore very much “work in progress” at this point in time.

Against this background, the following information emerged about impacts from the case studies described in Chapters 2 to 4. Omitted from consideration are what appeared to be purely general and anecdotal statements concerning the post-adoption effects of employing the biotechnologies concerned, which in any case were invariably beneficial. Unfortunately, this is how “people benefits” were framed in the great majority of cases, sometimes even in the absence of data actually demonstrating beneficial changes in yield, disease risk, product quality and safety, marketing, employment, income or other indices following adoption. Only the studies from West Africa (CS 4.5) and Thailand (CS 4.3) described – again in general terms – any of the possible adverse consequences arising from using a biotechnology. The former noted that the quality and safety of fermented fish products may be compromised by the lack of standardization of processing methods and hygiene during processing. The latter described how millions of interspecific hybrid catfish had escaped into natural water bodies during floods, leading to backcrossing with native catfish species, which may be contributing to the reduced fitness of local species. Also, the expansion of hybrid fish culture was reported to have resulted in the release of aquaculture wastes, causing mass mortality of aquatic animals and interrupting rice flowering due to excess nitrogen.

The paucity – even the absence – of hard data should certainly not be interpreted to mean that some or all of these biotechnologies did not improve incomes, reduce malnutrition, protect the environment or stimulate local, national or international trade. Indeed, on the scales at which they were adopted, it seems unimaginable that they did not positively affect the well-being of large numbers of farmers, their families and others in the wider population. Yet, in the

absence of appropriately collected and analysed data, conclusions about the significance of any effects – positive or negative – must remain speculative. Interestingly, the same general conclusion was reached from a study of non-transgenic biotechnologies (mainly tissue culture and micropropagation) in Africa (FAO, 2009).

There were, however, some noteworthy exceptions in terms of providing plausible evidence that the biotechnologies did “work” for producers and consumers:

- In 2011 alone, the improved pearl millet hybrids released in the states of Haryana and Rajasthan are estimated to have benefitted Indian farmers to the tune of US\$13.5 million more than local landraces, and seed production increased the net income of seed producers by US\$1 314 per ha or US\$6.4 million. Hybrid seed multiplication was estimated to have provided 900 000 person days of employment, nearly half of which were for women (CS 2.2).
- In Cuba, estimates of costs and incomes associated with using plantain produced by different methods showed that plants derived from somatic embryos produced a net profit per ha of \$62 Cuban pesos over those sourced from meristem cultures and a profit of almost \$8 000 Cuba pesos per ha over those derived from corm buds (CS 2.5).
- In Ghana, a three-stage strategy, including tissue culture, was used for the multiplication of sweet potato to produce clean planting materials. Almost 15 000 farmers who adopted and distributed two new resulting varieties had output increases and ready markets for their produce, and were very likely to have realized income gains (CS 2.3).
- In Sri Lanka, about 500 farmer families were involved in tissue culture banana cultivation. Farmers who switched from rice to growing mutant and other tissue culture virus-free bananas increased their incomes up to 25-fold (CS 2.1).
- In northern Patagonia in Argentina, the amount of certified quality mohair increased substantially from 1998 to 2006 and, through reforms set in motion by the government to strengthen smallholder organization, the price obtained for a bulk export order was 40 percent greater than the price that individual producers could obtain by selling individual lots of unclassified mohair (CS 3.3).
- In Zanzibar, the average monthly income of farming households increased by 30 percent between 1999 and 2002. In the same period, the proportion of households with an income over US\$25 per month increased from 69 percent to 86 percent and the proportion with an income over US\$50 per month increased from 22 percent to 36 percent. These changes can be associated with tsetse and trypanosomosis eradication since a strong correlation was observed between household income and milk yields, milk sales, and use of manure and animal power for cultivation and transport (CS 3.7).
- In the Satkhira district of Bangladesh, AI and other services provided through a community-based initiative increased the monthly incomes of most dairy farmers by between US\$1 and US\$19 per cow (CS 3.4).
- In the State of Maharashtra in India, the annual income from each ewe carrying the prolificacy *FecB* gene was around \$US34, while for non-carrier ewes it was US\$21.5 (CS 3.1).

Case study 2.4 from Nigeria provided “theoretical” estimates of benefit by carrying out *ex ante* impact economic assessments for introducing cassava cultivars with pest- and disease-resistance, as well as quality traits produced through marker-assisted breeding. Estimates indicated that over 20 years they might be worth US\$2.9 billion or, if developed only for pest- and disease-resistance, \$US1.5 billion. While undoubtedly useful for setting priorities between competing activities for research funds, and particularly for both delineating and monitoring of impact pathways during planning and implementation of activities, such studies cannot replace impact assessment carried out *ex post*.

LESSONS LEARNED

These 19 case studies, diverse as they are in terms of technology, sector, country and problem focus, and in the time periods over which they were operational, aim to provide readers with better insights into both the “what” and “why” of agricultural development achievements in developing countries using biotechnology. They also provide an opportunity for drawing general lessons for undertaking future interventions, while steering clear of defining what constitutes “success” or “failure”. This is important to stress, because the number of factors bearing upon both the nature and the size of any result is large, and some may be negative and others positive. Also, in the present context, while all studies referred to initiatives conducted outside laboratories, a sizeable number can best be described as being still at the experimental phase. In effect, they were part and parcel of the normal R&D process, allowing national staff to learn and adapt their strategies from small- to wider-scale interventions. To define these as successes or failures would clearly not be appropriate. That said, and aside from the admirable human qualities that were evident in all the case studies – technical and organizational leadership, long-term commitment, teamwork, and risk-taking – ten key and interrelated messages emerged about achieving outputs, outcomes and impacts. We look at these below.

NATIONAL AND STATE GOVERNMENT POLICIES WERE DIRECTED AT IMPROVING THE WELL-BEING OF SMALLHOLDER FARMERS

Each of the case studies illustrated efforts – often made under very difficult circumstances – to tackle one or a combination of the types of technical constraints faced by smallholder farmers in developing countries to produce more or better quality food using their indigenous crop and animal resources. The financial resources and regulatory environments within which they operated were largely determined by national exchequers, but were also supported by a variety

of external sources. While financial resources are always tight and some perceive regulation as too restrictive, the preceding chapters of this book demonstrate commitment on the part of national and/or state governments to focus on improving the productivity of smallholder enterprises and the livelihoods of those both directly involved and indirectly influenced. Probably the best illustrations of such commitments are provided by the case studies on aquaculture from China (CS 4.1 and 4.4); by the pearl millet, rice improvement and shrimp disease control projects in India (CS 2.2, 2.7 and 4.2 respectively), all of which were robustly supported by both the central and state governments and their regulatory agencies through specific crop improvement, poverty alleviation and climate change-related programmes; by the federal and various state governments in Brazil for supporting smallholder resettlement programmes (CS 3.5); by the government and various departments in Colombia with respect to cassava improvement (CS 2.6); and by the Government of Sri Lanka and its various ministries for banana micropropagation (CS 2.1).

FINANCIAL SUPPORT FROM BILATERAL AND MULTILATERAL DONORS AND FROM INTERNATIONAL AGENCIES WERE INDISPENSABLE FOR SUPPLEMENTING NATIONAL EFFORTS

The work on pearl millet (CS 2.2) was supported by the United Kingdom's Department for International Development (DFID) while the Bill and Melinda Gates Foundation currently supports the STRASA project (CS 2.7). In Ghana, work on sweet potato varieties was supported by the International Fund for Agricultural Development (IFAD), while work on plantain and banana hybrids was supported by the International Development Research Centre (IDRC), the United States Agency for International Development (USAID), the Gatsby Charitable Foundation of the United Kingdom and World Vision Ghana (CS 2.3). The development and use of PCR for shrimp diseases (CS 4.2) was assisted by FAO and the Australian Centre for International Agricultural Research (ACIAR), the latter of which also funded work to improve Deccani sheep in India (CS 3.1). Support for micropropagation in Sri Lanka (CS 2.1), the SIT in Zanzibar (CS 3.7) and LAMP in Cameroon (CS 3.6) came from the Joint FAO/IAEA Programme in Vienna, together with a number of international and bilateral donors including the Common Fund for Commodities, IFAD, the Organization of the Petroleum Exporting Countries (OPEC) Fund for International Development, and national governments (Belgium, Canada, China, Sweden, the United Kingdom and the United States).

All the biotechnologies discussed here were discovered many decades ago, and in the interval have undergone many innovations both in industrialized and in developing regions to

improve their performance and widen their application. Although, again, specific information is not available from most case studies (the Indian pearl millet (CS 2.2) and Sri Lankan banana (CS 2.1) studies being notable exceptions), it is highly likely that all the case studies benefitted from financial support for direct or indirect scientific exchanges through workshops, courses, training fellowships etc. under the great variety of programmes associated with intergovernmental and institutional agreements.

INTERNATIONAL AND NATIONAL PARTNERSHIPS WERE VITAL FOR ACHIEVING RESULTS, PARTICULARLY FOR TRANSLATING RESEARCH OUTPUTS INTO FIELD OUTCOMES AND IMPACTS; SOME INVOLVED NEW ORGANIZATIONAL MEASURES OR STRUCTURES

These case studies provided numerous examples of partnerships both within the public sector and involving international and national collaboration, as well as partnerships between public and private sector entities. For example, all but one of the crop studies described the involvement of specific CGIAR centres, such as Bioversity International, CIAT, ICRISAT, IITA and IRRI. Notable examples of partnerships in the case studies include:

- In India, the partnerships between ICRISAT, national agricultural universities such as the Chaudhary Charan Singh Haryana Agricultural University (CCSHAU) and advanced research institutes in the United Kingdom ultimately led to the releases of pearl millet varieties, began with ground-breaking work to develop DNA markers, establish genetic linkage maps, map populations and to use MABC. ICRISAT, CCSHAU and others then performed the field trials needed for government approval. The Department of Agriculture and Cooperation, seed producers and suppliers then took on responsibility for distributing seed to farmers. This was a fine example of biotechnology at work through a “value-chain” partnership approach that involved fruitful interaction between science, technology and government policy (CS 2.2).
- Also in India, an extensive network of partnerships, involving IRRI, CRRRI, NDUAT and other stakeholders from the public and private sectors, NGOs and farmers’ organizations, were built through the STRASA project to develop and widen the benefits of submergence-tolerant rice to other countries and regions. These, together with innumerable trials conducted by research stations, were critical for obtaining the approval of state governments in India to produce seed, create demand and release improved rice lines to farming communities (CS 2.7).
- Partnerships were also established between institutes within the Chinese Academy of Fishery Sciences, the Ministry of Agriculture and both its extension and regulatory departments, sales companies, demonstration farmers and smallholder producers for developing and marketing probiotics for shrimp farming. Again, regulatory decision-making was influenced, in that China was able to start re-exporting shrimp to the European Union after the lifting of a ban originally imposed because of unacceptable levels of antibiotic residues (CS 4.1).

- The partnerships in developing, standardizing, quality assuring and using PCR tests between fisheries research institutes, private agencies, hatcheries and the Marine Products Export Development Authority (MPEDA) in India which, among others, is responsible for specifying standards for producing, processing and trade in aquaculture, influenced national policy-making with respect to tests, standards and disease control measures (e.g. quarantine procedures). These were outcomes of significant national importance (CS 4.2).
- Partnerships involving NGOs and community-based approaches were evident in several case studies, some bringing positive organizational changes and new structures. These are best exemplified by:
 - The community-based micropropagation facilities for cassava established in Colombia through partnerships between CIAT, the women's farmers group from Santa Ana (ASOPROSA), an NGO (Fundacion para la Investigacion y Desarrolla Agricola) and numerous other local partners (CS 2.6).
 - Establishment of the Community-based Dairy Veterinary Foundation (CDVF), involving the NGO called BRAC, the Bangladesh Agricultural University, farmers' associations, veterinary service providers and milk processors, to deliver production-related veterinary services to smallholder farmers in four dairy-producing areas of Bangladesh (CS 3.4).
 - The Nimbkar Agricultural Research Institute (NARI) providing smallholders with more prolific and productive sheep (CS 3.1).
 - The collaboration between the Brazilian Agricultural Research Corporation (EMBRAPA), a local cooperative (COOASGO) and a local company (Retificadoro Centro Sul) to support the Campanário Settlement (CS 3.5).
 - The linkages established between the National Institute of Agricultural Technology (INTA) and the Mohair Programme established by the Government of Argentina to strengthen collective action by goat farmers (CS 3.3).
 - The contract hatchery system and establishment of farmer societies and aquaclubs promoted by MPEDA and the Network of Aquaculture Centres in Asia-Pacific (NACA) to promote sustainable shrimp rearing and marketing in India (CS 4.2).

LONG-TERM NATIONAL INVESTMENTS IN BOTH HUMAN CAPITAL AND INFRASTRUCTURE FOR SCIENCE AND TECHNOLOGY WERE CRITICAL – BUT NOT SUFFICIENT COMPONENTS OF THE RECIPE

None of the cases detailed the financial inputs but, with the exception of those from Brazil, Cameroon and South Africa (CS 3.5, 3.6 and 3.2, respectively) and excluding knowledge and products accumulated over previous years, all entailed continuous AR4D efforts that extended over 15-40 years. This is consistent with the accumulation of steady and incremental innovations and benefits from the technologies pursued rather than from rapid and revolutionary change, although innovative products did reach beneficiary groups substantially quicker than would normally be expected through other approaches.

BIOTECHNOLOGY APPROACHES DID NOT WORK IN A VACUUM – THEY WERE INTRODUCED INTO BOTH THE RESEARCH MIX AND FARMERS’ FIELDS AND PONDS THROUGH APPROPRIATE INTEGRATION WITH OTHER SOURCES OF SCIENCE-BASED AND TRADITIONAL KNOWLEDGE

This was evident from all the case studies. For example, all results using molecular markers required sound knowledge of how to select parents, make crosses and backcrosses and then select improved plants or animals. The same applied to producing hybrid catfish or carp by gynogenesis. All these biotechnologies required a sound understanding of traditional procedures for plant, animal and fish selection and breeding. Tissue culture is a relatively straightforward technique but also requires an understanding of the “basics” – the physiology, anatomy and nutritional requirements of the plant species being propagated, as well as the skills actually to grow plants in an incubator and “harden” them in a nursery before providing them to farmers. Likewise, PCR tests for animals are useful only if the people using them know about the clinical features and epidemiology of the diseases concerned – skills and knowledge that, again, can come only from a solid education in veterinary medicine. Conventional, and particularly laparoscopic, AI also requires traditional breeding, anatomical, physiological and surgical skills. There is hardly any point in producing high-quality semen if the knowledge and skills are insufficient to select males with the appropriate genetic background, to detect whether recipient females are “in heat”, synchronize oestrus or inject semen into the uterus. Similarly, releasing sterile insects in the field needs sound knowledge of insect behaviour and habitat to determine where to lay simple traps and targets to attract wild flies and release sterile ones, all of which are helped by geographical information systems (GIS). CS 2.7 on submergence-tolerant rice is a further illustration of the critical importance of “technology targeting”, in this instance using GIS and “ground truthing” to identify areas and villages affected by floods for prioritizing activities for seed distribution according to greatest need.

In no case would any of the accomplishments described have been possible without the knowledge, skills and support of the targeted smallholder groups themselves. The case studies from Colombia, Ghana, India and Sri Lanka (CS 2.6, 2.3, 4.2 and 2.1 respectively), in particular, emphasize “farmer participatory approaches” with respect to problem identification, technology development and translation.

In short, there are no quick fixes, and no room for piecemeal approaches for agricultural biotechnologies. As demonstrated by these case studies, biotechnologies need to be

incorporated into technological mixes whose foundations lie in traditional scientific knowledge, skills and approaches, with decisions concerning both their real need and utility being determined through participation with the ultimate end beneficiaries.

THE DIFFUSION OF GENETIC RESOURCES, TECHNIQUES AND KNOW-HOW ACROSS NATIONAL AND CONTINENTAL BOUNDARIES WAS AN ESSENTIAL INGREDIENT OF MOST CASE STUDIES

Few of the case studies described the origins of the plant and animal genetic resources used, although significant transfers of germplasm took place across continents and individual countries. These were in the form of rice landraces, pure line selections and varieties (within India and more widely between Asian countries); cassava plantlets (from Colombia to Nigeria); plantain and banana plantlets (from Honduras to Cuba and Ghana); probiotic bacteria strains into China from Japan, Sweden and the United States; cattle semen in Bangladesh; and even of live animals, such as the African sharp-tooth catfish into Thailand from Vietnam and *P. vannamei* specific pathogen-free shrimp from other countries in Asia into India. In the cases of both cassava and shrimp, it was only through developments in the biotechnologies described that such transfers became possible.

No case study mentioned that arrangements regarding access to and the use of genetic resources were difficult to conclude, which suggests that the institutes and countries concerned honoured the principles of unrestricted access and prior informed consent, and made such arrangements through material transfer agreements (MTAs).

INTELLECTUAL PROPERTY ISSUES NEITHER CONSTRAINED RESEARCH FOR DEVELOPMENT NOR THE PRODUCTION OR USE OF BIOTECHNOLOGY INNOVATIONS

The issue of intellectual property rights (IPR) did not appear on the “radar screen” of any case study either as a source of hindrance or of finance to promote the biotechnologies concerned. This suggests that despite the existence of patents and other forms of intellectual (e.g. MTAs) and tangible property rights covering specific components and “tricks of the trade” in using essentially all of the biotechnologies in the studies described, the institutions concerned appeared to have “freedom to operate”. The possible reasons are many, and are outlined in Chapter 9 of FAO (2011a). In the absence of specific information (apart from CS 2.2 which

noted that the John Innes Centre waived the IPR to the RFLP markers used for downy mildew resistance in pearl millet), the case studies did not mention how the technologies concerned moved across national borders.

PRODUCTS GENERATED THROUGH BIOTECHNOLOGIES DID NOT CONFORM TO SPECIFIC BIOSAFETY AND FOOD SAFETY REGULATIONS OR STANDARDS

None of the case studies indicated that the processes and products from the biotechnologies that were developed and used required new national laws and regulations covering R&D, sanitary (human and animal) and phytosanitary (plant) measures, often referred to as “biosecurity” measures (FAO, 2007). New technical rules such as labelling appeared also not to be required. While not suggesting that national regulations (e.g. on variety release, milk or mohair quality, antibiotic residue levels etc.) played no part in determining the timeframe from product development to field, hatchery or pond release, this contrasts with the additional regulatory requirements imposed by national authorities on genetically modified organisms. Nevertheless, as the case studies on shrimp and hybrid catfish (CS 4.1, 4.2 and 4.3) illustrate well, sudden changes in water levels and quality – irrespective of the technology concerned – can dramatically increase the biosecurity risks associated with intensive aquaculture. Also, CS 4.2 warned about the possibility of introducing new pathogens and diseases through the importation of new species and the future challenges in developing biotechnological tests for diagnosing such emerging diseases. The adverse consequences of some of these hazards may be long-term.

THE “GOALPOSTS” CHANGED, REQUIRING BOTH FORESIGHT AND FLEXIBILITY

Both the dynamic and risk-prone nature of producing and marketing food arising from changes in farmer and consumer preferences or field breakdown of desirable traits is illustrated by three of the case studies, namely those dealing with drought-tolerant and disease-resistant pearl millet (CS 2.2), Deccani sheep breeding (CS 3.1) and shrimp disease diagnostics and control in aquaculture (CS 4.2). In the first of these, the HHB 67 variety had to be gradually replaced after less than 20 years by HHB 67 Improved, which had greater resistance to DM, and even the advantages of this new hybrid need to be continually secured by maintenance breeding to enhance the downy mildew resistance of its parents. In the case of the Deccani sheep, farmers changed their preference for rams of the Madgyal breed because crossbred lambs of the latter

grew much faster. In the third study, the replacement of *P. monodon* with *P. vannamei* farming occurred because of the faster growth and lower cost of production and also because of the availability of specific pathogen-free stocks of *P. vannamei*.

Smallholder farmers already face an uphill battle in increasing the productivity and sustainability of their holdings to feed their families and improve their livelihoods. In the fast-changing world of the twenty-first century, agriculture and food production take place against the backdrop of climate change, ever more competitive food and commodity markets and national and global policies. Transforming smallholder production systems into sustainable business-oriented farming will consequently require greater foresight and flexibility on the part of all stakeholders to anticipate and provide locally specific technical, institutional and policy solutions for the evolving realities of such systems.

PLANNING, MONITORING AND EVALUATION OF BIOTECHNOLOGY APPLICATIONS WERE WEAK AND ARE IN URGENT NEED OF STRENGTHENING

With the exception of those involving LAMP and the SIT (CS 3.6 and 3.7 respectively), none of the case studies provided information about costs, although these can be quite substantial in the case of technologies such as tissue culture and micropropagation, developing molecular markers, AI, cryopreservation and the SIT. In fact, as illustrated by the cases of micropropagation in Ghana (CS 2.3) and the SIT in Zanzibar (3.7), it is highly questionable whether these technologies could be sustained without generating income from product sales or without the support of outside agencies. In essentially all cases, the fruits of AR4D were provided free of charge to the farmers or countries concerned. Also, most studies provided no information concerning the benefits in terms of production, productivity or financial returns, and plausible evidence about livelihood changes was conspicuous by its scarcity. The point here is that notwithstanding the value of collecting and analysing changes in yields and/or in costs and benefits in purely economic terms, a more comprehensive or multidimensional approach that supplements such traditional indices of agricultural research performance is needed to assess the true benefits or costs of agricultural interventions at the household or village level or on a larger scale.

One example from these case studies illustrates this point: the improvement of the lives of women farmers or casual labourers by providing employment through plant tissue culture and micropropagation in Colombia and Sri Lanka (CS 2.6 and 2.1 respectively). How should this be measured to track progress in closing the gender gap – an aim which in the wider

sense is essential for increasing agricultural productivity, reducing hunger and achieving food security at household, community and even national levels (FAO, 2011b)? Tools such as the women's empowerment in agriculture index (WEAI) are now becoming available. WEAI is based on household interviews, and provides an aggregate index based on defined domains for employment and sets of indicators with binary scores for each (Alkire *et al.*, 2013).

Tools designed to capture the linkages between AR4D, nutrition and the environment are also being developed and piloted using essentially the same approach and some of these, together with their application, are highlighted on the CGIAR's SPIA web site (<http://impact.cgiar.org/about>). The point here is that there is no substitute for including careful impact assessment of biotechnology applications in food and agriculture, and one can only hope that for the sake of improving both the planning and management of future projects, countries and their institutions will now give this much higher priority than in the past.

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This book documents a unique series of 19 case studies where agricultural biotechnologies were used to serve the needs of smallholders in developing countries. They cover different regions, production systems, species and underlying socio-economic conditions in the crop (seven case studies), livestock (seven) and aquaculture/fisheries (five) sectors.

Most of the case studies involve a single crop, livestock or fish species and a single biotechnology. The biotechnologies covered include some that are considered quite traditional, such as fermentation and artificial insemination, as well as other more modern ones, such as the use of DNA-based approaches to detect pathogens.

Prepared by scientists and researchers who were directly involved in the initiatives, the authors were able to provide an insider's guide to the background, achievements, obstacles, challenges and lessons learned from each case study. The final chapter of the book summarizes the background, challenges, results and lessons learned from the 19 case studies.

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