Case studies of the use of agricultural biotechnologies to meet the needs of smallholders in developing countries

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Abbreviations

AATF	African Agricultural Technology Foundation
AGI	Agricultural Genetics Institute (Viet Nam)
AI	artificial insemination
ASF	African swine fever
BMGF	Bill and Melinda Gates Foundation
Bt	bacillus thuringiensis
CIMMYT	International Maize and Wheat Improvement Center
CS	case study
DNA	deoxyribonucleic acid
EFSB	eggplant fruit and shoot borer
ELISA	enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization of the United Nations
FAW	fall armyworm
GAB	genomics-assisted breeding
GEF	Global Environment Facility
GM(O)	genetically modified (organism)
IAEA	International Atomic Energy Agency
ICAR	Indian Council of Agricultural Research
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IFGTB	Institute of Forest Genetics and Tree Breeding (India)
IFPRI	International Food Policy Research Institute
IPM	integrated pest management
MABC	marker-assisted backcrossing
MARS	marker-assisted recurrent selection
MAS	marker-assisted selection
NARS	national agricultural research systems
NGO	non-governmental organization
PCC	Philippine Carabao Center
PCR	polymerase chain reaction
PPP	public–private partnership
QTL	quantitative trait locus
R&D	research and development
RPS	relative percent survival
SIT	sterile insect technique

SNP	single nucleotide polymorphism
SSA	Sub-Saharan Africa
SSR	simple sequence repeat
SSS	sex-sorted semen
UN	United Nations
USAID	United States Agency for International Development
USD	US dollars

Chapter 1: Introduction

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Background

There are tremendous challenges facing the world's food and agriculture systems today. In 2021 an estimated 702–828 million people in the world were undernourished, meaning that their habitual food consumption was insufficient to provide, on average, the amount of dietary energy they require to maintain a normal, active and healthy life (FAO *et al*., 2022). Even when people have access to enough food for their energy needs, it may not always provide them with all the vitamins and other nutrients that they require. Over 3 billion people were unable to afford a healthy diet in 2020 (FAO *et al*., 2022). Thirty percent of women of reproductive age globally were affected by anaemia in 2019. Problems relating to overweight and obesity also continue to increase worldwide. For example, adult obesity almost doubled globally from the year 2000 (343 million people) to 2016 (676 million) (FAO *et al*., 2022).

In the future, the demand for food is also expected to substantially increase as the global population increases and as higher incomes drive dietary pattern changes towards more livestock products. The agriculture sectors, including forestry and fisheries, are also expected to produce more non-food products for energy and feed. At the same time, the natural resources upon which agriculture depends – such as land, water and soil – are finite and increasingly threatened by environmental degradation and climate change.

Climate change has already begun to significantly affect agriculture. Temperatures, rain patterns, water availability, sea levels and salinization, as well as the frequency and intensity of extreme weather events, are all changing, with profound impacts on the crop, livestock, forestry and fishery sectors.

Looking to the future, it is imperative that our food systems are transformed so that they produce more food that is also of greater nutritional value, and that they do this with less environmental damage and in the face of climate change.

FAO is working with its Member Nations and development partners to reverse the current trend of worsening food insecurity and malnutrition as a means to achieve the Sustainable Development Goals (SDGs), especially SDG 2 (to end hunger, achieve food security and improved nutrition, and promote sustainable agriculture), by 2030. FAO's work is guided by its new Strategic Framework, which seeks the transformation to more efficient, inclusive, resilient and sustainable agrifood systems for better production, better nutrition, a better environment and a better life, leaving no one behind (FAO, 2021a).

Agricultural biotechnologies

Agricultural biotechnologies are an integral part of the "toolbox" that can be used for achieving the transformation of food systems. FAO's definition of biotechnology is based on that of the Convention on Biological Diversity and is quite broad. It encompasses a wide range of low- to high-tech tools such as artificial insemination, tissue culture and fermentation techniques, as well as whole genome sequencing and approaches based on polymerase chain reaction (PCR) for disease diagnosis and monitoring (FAO, 2011).

Tremendous advances have been made in biological sciences in recent years, allowing us to broaden our understanding of complex mechanisms in plants, animals and micro-organisms. Building on this knowledge, agricultural biotechnologies are increasingly recognized as key emerging technologies, like digital technologies, which can play an integral role in helping countries to achieve the muchneeded increases in food production that are required in the future without increasing the deleterious environmental footprint of the agrifood system.

The importance of FAO's role in facilitating knowledge sharing and in acting as a neutral broker regarding agricultural biotechnologies has been underlined on many occasions by FAO's governing and statutory bodies. For example, its main governing body, the FAO Conference, which normally meets every two years, convened for its 41st Session on 22–29 June 2019 and adopted Resolution 7/2019 which, *inter alia*, requested FAO to assist countries and regions to enhance their commitment and engage more effectively in transitioning towards sustainable agriculture and food systems by "encouraging innovation in agriculture, *inter alia*, through the utilization of relevant and context-adapted technology and tools – including ICT and biotechnology – as it can offer a solution to a wide range of issues" (FAO, 2019). More recently, the Commission on Genetic Resources for Food and Agriculture, at its 18th Regular Session on 27 September to 1 October 2021, requested that FAO "continue to review traditional, maturing and emerging biotechnologies for the characterization, sustainable use and conservation of genetic resources for food and agriculture" (FAO, 2021b).

Documenting case studies of agricultural biotechnologies

The documentation of the application of agricultural biotechnologies for smallholders is an important part of FAO's role in facilitating knowledge sharing regarding agricultural biotechnologies. In this role, FAO has carried out a number of initiatives in recent years. In 2009, FAO moderated an email conference on "Learning from the past: Successes and failures with agricultural biotechnologies in developing countries over the last 20 years". Over 800 people participated in the email conference, which lasted one month. They shared a wealth of experiences regarding the use of agricultural biotechnologies across the different food and agricultural sectors in developing countries. They also provided concrete examples where agricultural biotechnologies were benefiting smallholders in developing countries (FAO, 2011).

The email conference took place as part of the lead up to the FAO international technical conference on 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) which took place in Guadalajara, Mexico on 1–4 March 2010 (FAO, 2011; Ruane and Sonnino, 2011). During this conference, five parallel sessions were dedicated to case studies of what were generally considered to be successful applications of biotechnologies in developing countries.

There was one session each dedicated to livestock, crops, fisheries, forestry and agro-industry respectively. For every session, two to three case studies were presented, followed by a facilitated discussion, providing attendees with an opportunity to evaluate the key factors responsible for the results obtained (FAO, 2011).

Building on the success of these parallel sessions, FAO decided in 2012 to open a widely disseminated call for people and organizations to submit case studies where biotechnologies had been applied to serve the needs of smallholders in developing countries. From these, 19 case studies were selected covering the crop, livestock and aquaculture/fisheries sectors. They included 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 (Ruane *et al.*, eds., 2013).

In 2016, FAO hosted the International Symposium on the "Role of Agricultural Biotechnologies in Sustainable Food Systems and Nutrition", which was the first major conference dedicated to biotechnology at FAO headquarters (Ruane, Dargie and Daly, eds., 2016). In 2017, FAO hosted two follow-up regional meetings in Asia-Pacific (FAO, 2018a) and sub-Saharan Africa (FAO, 2018b). For the regional meeting in Asia-Pacific, FAO also prepared videos of four different case studies in the crop, livestock, forestry and fishery sectors, and these were shown in the plenary sessions of the meeting.

For these three meetings, the previous work carried out on documentation of the case studies was fundamental for their success. Biotechnology is a controversial topic, and many people see biotechnology and genetically modified organisms (GMOs) as being one and the same. In all the information materials for these three meetings, FAO provided many useful examples from the case studies clearly demonstrating that:

- biotechnology is much more than GMOs and encompasses a wide range of biotechnologies, including those that are low-tech as well as high-tech;
- biotechnology is not just for rich countries or big farmers, but is also being used successfully for smallholders in developing countries; and
- despite the complexities of smallholder farmer production systems, agricultural biotechnologies can represent powerful tools to benefit smallholder farmers, given the appropriate conditions and enabling environment.

To build on, complement and update this work on the application of agricultural biotechnologies for smallholders, FAO opened a second call for case studies from April–June 2022, ten years after the first open call. Stakeholders worldwide were encouraged to submit an abstract (of a maximum of 300 words) describing case studies where agricultural biotechnologies were applied to solve the problems of smallholders in developing countries in the aquaculture, crop, forestry and livestock sectors. The call was widely circulated both within and outside FAO. The best abstracts out of nearly 100 submissions were chosen, after which the authors were invited to prepare a more detailed description of their case study.

In selecting the best abstracts, appropriate attention was given to regional representation as well as to ensuring that they covered a wide range of agricultural biotechnologies in addition to the different purposes for which agricultural biotechnologies are applied, such as to increase productivity and quality, characterize and conserve genetic resources and diagnose or prevent diseases.

There are a wide range of biotechnologies available. Many are currently being used in situations and sectors worldwide to solve the myriad problems that farmers are facing. As such, these case studies merely represent the tip of the iceberg. In addition, the tools and approaches continue to evolve and advance, and so, in addition to the use of biotechnologies in a specific place and context, the case studies also represent a snapshot of their use at a specific time. It is nevertheless the hope that these case studies will provide a source of inspiration and encouragement for those wishing to apply biotechnologies to serve the needs of smallholders, as well as providing a source of well-documented case studies from which to learn. The 15 case studies are presented in Chapters 2 and 3, while Chapter 4 presents a brief overview and some reflections on the case studies.

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Chapter 2: Case studies in livestock and aquaculture

2.1 Use of artificial insemination in dairy farming to improve milk production and smallholder farmers' livelihoods in the southern region of Malawi

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Background

In Malawi, milk and milk products play a very important role in income generation for smallholder farmers and in nutritional security for the population at large. However, production has always lagged behind demand since pre-colonial times when farmers used the indigenous Malawi Zebu as the main source for milk. The average Malawi Zebu cow produces 2–3 litres of milk per day. The urbanization of Zomba as the British Central African Protectorate's first capital in 1891 and the attainment of municipal status of Blantyre in 1895, coupled with general population growth across the southern region, led to a rapid increase in demand for milk in Malawi.

Earlier efforts to meet this demand were made by colonial farmers in southern Malawi. From 1952 to 1954 they imported more than 50 Friesian cattle from South Africa and Zimbabwe. They sold the milk directly to consumers (Munthali, Musa and Chiwayula, 1993). Many smallholder farmers in southern Malawi were also interested in dairy farming. However, the traditional Malawi Zebu could not provide enough milk to make milk sales economically viable. The farmers also could not keep large herds, as land holdings in this region were small, averaging 0.4 hectares. There was thus a need to increase animal milk productivity in order to improve the livelihoods of farmers who depended on milk sales. The use of high milk yielding animals was one possible solution to improving milk productivity without increasing land size. This case study describes how the application of artificial insemination (AI) using superior breeds like the Holstein/Friesian heifers improved the milk yields of Malawi Zebu, and ultimately improved people's livelihoods in the Shire highlands of southern Malawi.

In the 1960s, the Malawi Government recognised the importance of milk and milk products for nutrition, and the income opportunity it offered smallholder dairy farmers. However, widespread intensive smallholder dairy production in Malawi only began in 1969 under a government initiative with assistance from FAO when farmers within an 8 to 10 km radius were organised into milk bulking groups (MBGs) to operate as milk collection and checking centres. Each MBG was equipped with a

milk cooling tank. This led to the establishment of the first processing plant in Blantyre through the Malawi Milk Marketing Project in the same year.

The distribution of imported dairy animals started around 1979 when the Government of Malawi, in partnership with the Canadian International Development Agency (CIDA), imported the first 400 Holstein-Friesian heifers from Canada and distributed them across the three regions in the country (Baur *et al.*, 2017). These efforts were later complimented by organizations like Land O'Lakes, the Small Scale Livestock and Livelihoods Program (SSLPP), World Vision Malawi and Heifer International under a pass-on programme where a farmer received a heifer and the first female calf born from each beneficiary was given to a subsequent beneficiary.

Continual importation of heifers seemed unsustainable. As a result, the government, in collaboration with development partners, introduced AI services to improve the milk production of the Malawi Zebu through crossbreeding. Artificial insemination in dairy animals is a technology in which semen is collected from a bull and manually deposited into the cow's uterus so that it can conceive. If the bull is from a genetically superior dairy breed, it is expected that the crossbred daughters will give higher milk yields than the Malawi Zebu dam.

When AI was introduced in Malawi, all semen used was imported from the United States of America. As the number of animals increased, resulting in more demand for AI services, the government established the National Artificial Insemination Centre (NAIC) at Mikolongwe livestock farm in the southern region in the early 1980s. The first bulls used for semen collection were from Europe and New Zealand. Local semen collection was introduced at NAIC in 1984 and, by 1989, locally produced semen was used in the smallholder sector (Mwenifumbo, 1995). The one Jersey and two Friesian bulls currently being used were sourced locally. These bulls were not progeny tested, but were instead selected using their phenotype.

NAIC is involved in the collection, processing, preservation and utilization of semen from exotic dairy breed bulls. The centre has trained a team of livestock staff on the heat detection, collection and processing of fresh semen from exotic bulls, and how to perform AI. So far, NAIC has trained 550 AI technicians. The centre currently produces around 31 200 semen straws per year which are used in the southern region as well as the rest of the country. As of 2022, NAIC remains the only AI centre in the country.

Currently, AI is conducted when farmers report cows on heat. There are 124 practicing AI technicians along the Shire Highlands of southern Malawi conducting approximately 25 200 AI services annually, and charging a service fee of MK 7 500 (USD 7.50) per animal. One beneficiary of AI service training is Lucius Majiya (Figure 1), a 47-year-old farmer who was trained under the Japanese International Cooperation Agency (JICA) funded "Farmer Artificial Insemination Technician Foster Project". As part of the training package, he was provided with a full AI equipment set, comprising a bovine AI gun, portable liquid nitrogen flask, forceps, straw cutter, thermometer and a bicycle for mobility, so that he can visit and serve dairy farmers in his community. He has been practicing AI for over 21 years and is highly experienced. To date, he has carried out more than 7 052 inseminations (including repeat breeding) and he has produced over 3 000 calves. He said that, under normal circumstances, his conception rate is between 60–70 percent, but the pregnant animals are frequently sold before they give birth. Mr Majiya regards AI as a profitable activity, since it brings him income. He has also managed to build a house and buy a motorcycle. He charges MK 7 000 (USD 7) per insemination, while the cost of semen is MK 2 500 (USD 2.50).

Figure 1. Mr Majiya, a farmer AI technician who earns a living through AI service provision, standing at the NAIC

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Over the past decade, NAIC semen production has increased (Figure 2) due to higher demand because of a greater number of dairy animals in the country.

Figure 2. Trend in doses of semen produced by NAIC 2012–2021

NAIC semen production trends over 10 years
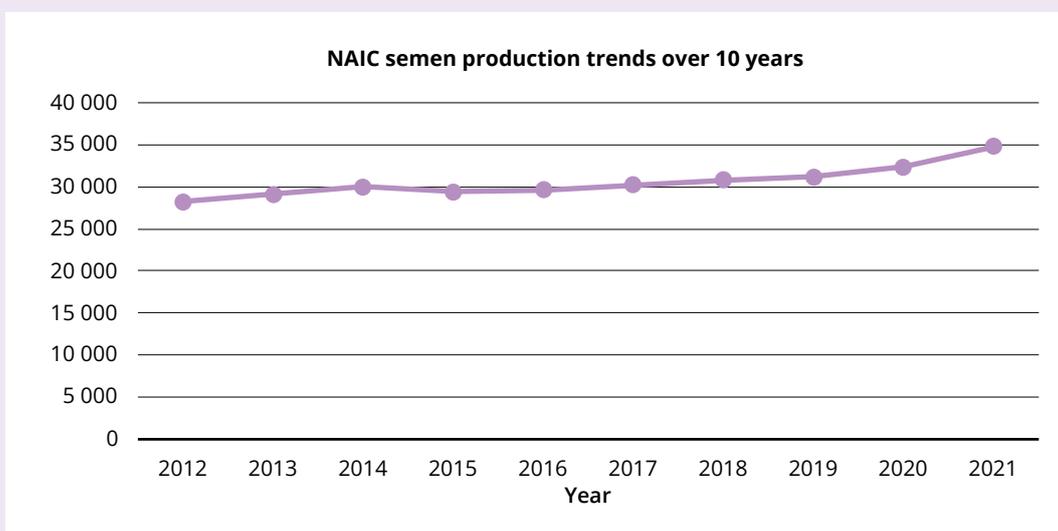
The AI technology has been well received and adopted by the target group, the smallholder dairy farmers, especially in the Shire highlands. With one bull, one can service many cows. The technology also reduces the costs of feeding and maintaining exotic bulls.

In 1985, as the number of MBGs increased in the southern region, the Shire Highlands Milk Producers Association (SHMPA) was formed (Chagunda *et al.*, 2004) to oversee smallholders' dairy farming and management activities in southern Malawi. There are now about 80 MBGs with 20 500 dairy farmers under the SHMPA owning 61 700 animals, representing 50 percent of all dairy farmers in Malawi. The total milk marketed per day is about 142 000 litres representing 80 percent of the total milk produced and marketed in the country. These MBGs also facilitate farmer training and advisory services.

On average, an individual farmer owns around 1.5 to 3 dairy cows, each producing 8 to 15 litres of milk per day, an increase from 2 to 3 litres per day for the pure Malawi Zebu. A detailed economic analysis of the operations of a typical experienced farmer who uses AI, carried out in February 2023 using real farm data, estimated that the farmer had net earnings of about USD 1.71 from milk sales per milking cow per day. The analysis assumed that ten cows were milked for 305 days in the year, the average daily yield was 14 litres and the milk price per litre was MK 250 (USD 0.25). The estimated annual revenue from milk sales was therefore MK 10.675 million. Regarding expenditures, the farmer hired four farm labourers (each paid MK 0.24 million annually for a total of MK 0.96 million), five boys to bring two bundles of grass per day (each costing MK 200 per bundle, meaning an annual cost of MK 0.73 million). The farmer also supplemented the animal feed with maize bran (20 bags per month, each costing MK 10 500, for a total of MK 2.52 million), used AI (two services on 10 cows, each costing MK 7500, for a total of MK 0.15 million) and paid MK 0.075 million for other items, including veterinary medicines. With a total annual revenue of MK 10.675 million and annual expenditures of MK 4.435 million, the farmer's total estimated net profits from milk sales were therefore MK 6.24 million (USD 6 240).

The milk is sold through MBGs where processors like Lilongwe Dairy collect the milk every two days. Lilongwe Dairy has a large market share of about 70 percent of the total marketed milk in the southern region and the country as a whole. Currently, milk is being sold at MK 250 per litre (USD 0.250 per litre) at MBGs.

Farmers that do not belong to an MBG, due to the unavailability of cooling centres in their area, sell their milk through informal markets directly to consumers at MK 350 (USD 0.35) per litre. Although informal markets offer higher prices, the formal markets offer a more consistent income source for farmers within the MBGs. A readily available market for the milk has made dairy farming in southern Malawi successful and economically viable. As a result, these MBGs have facilitated business development within their communities. Support services along the dairy value chain have created employment for many, especially in AI service provision and feed supplies.

One proud beneficiary who has seen success in dairy farming is Mrs Agness Kunena (Figure 3) who ventured into dairy farming in 2002 after the death of her husband. She is now a proud owner of 15 dairy animals (four heifers, ten cows and one bull) and three cooling centres, and has managed to build herself a modern house, educated her three children and bought herself six hectares of land where she now grows different crops. She received her first dairy cow in 2002 through a SHMPA project called Mkakazi which supported women farmers through a pass-on programme.

Dairy farming is labour intensive. If a farmer chooses to use a zero-grazing system, the animals must be fed twice a day. About 90 percent of animals in the total dairy herd are kept under a

Figure 3. Mrs Agness Kunena, proud owner of 15 dairy animals

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zero-grazing system while a few are grazed or raised on a mixed farming system. The need to feed dairy animals under a stall feeding regime has created an income generating opportunity within the communities. A number of individuals collect about 15–25 kg of fresh grass each and sell them to dairy farmers at MK 150–200 (USD 0.15–0.2). Andrew Alfred (Figure 4) is one such young man who supports his family through collecting and supplying grass to dairy farmers. The additional benefit of dairy farming is the improvement of soil fertility through the collection and application of manure onto farmers' crop fields, leading to higher crop yields thereby reducing application of expensive synthetic fertilizers. Farmers get USD 2.5–3.0 for 50 kg of manure.

Obstacles and challenges

The fundamental problem with dairy development in Malawi is the limited number of high-producing dairy animals. It relies heavily on the importation of heifers and semen from countries like Canada and the United States of America. The SHMPA and Malawi Milk Producers Association import semen and distribute it to farmers and AI technicians. Imported semen accounts for almost 50 percent of the country's semen market share. The introduction of germplasm through AI to enhance the production potential of the Malawi Zebu breed decades ago has been one of the quickest and most efficient ways of improving the productivity and reproductive performance of the dairy herd. However, its impact on milk production remains far below the government's expectations as the supply has not yet satisfied the local demand as evidenced by the current average consumption of milk in the country which is 5 kg per person per year, way below an African average of 15 kg per person per year (Ministry of Agriculture, 2020).

Figure 4. Mr Andrew Alfred, earning a living through collecting and selling grass to dairy farmers

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The annual number of inseminations per AI technician is also very low with an average of 175 per year, suggesting low efficiency of the programme resulting in only 10 percent of the country's herd being bred by AI. The other problem is irregular and interrupted supply of liquid nitrogen and other AI consumables. In addition, NAIC is the sole producer of liquid nitrogen for this purpose in the country. Other institutions that produce liquid nitrogen include the John Hopkins University Research Unit at the Queen Elizabeth Central Hospital, the Malaria Project of the College of Medicine, the Centre for Tick and Tick-Borne Diseases and the University of North Carolina Project-Malawi (UNC Project) in Lilongwe. However, these institutions produce liquid nitrogen for their own use. This has resulted in a further reduction in the number of AI services offered to farmers by AI technicians, which has led to reduced income from the service and use of bulls. Therefore, the AI service is likely to remain poor and could even decline due to inconsistent service delivery to dairy farmers. Furthermore, dairy cattle are kept in low input production systems, as they are largely fed grass, crop residues and supplemented with maize bran, resulting in low milk yields.

Lessons learned

The dependance on the importation of semen and live animals of exotic breeds to improve milk production and productivity has proved to be unsustainable in Malawi. There is a need to maintain a collection of individuals from high yielding breeds in the country at all times. The quality of AI services is declining because of difficulties in accessing liquid nitrogen. There is a need for consented efforts to be made in improving availability and access to semen and liquid nitrogen.

Conclusion

The introduction of high-yielding breeds of cattle and AI services in Malawi has effectively laid the foundation for an agricultural activity that has grown to be a major source of livelihood for smallholder dairy farmers in southern Malawi.

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2.2 The benefits of sex-sorted semen for smallholder dairy farmers in India

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Background

India continues to be the largest milk producer in the world, producing 210 million tonnes from 2020–21. Several measures have been initiated by the government to increase the productivity of the individual dairy animals, which can be witnessed by annual growth of 5.8 percent in milk production (Department of Animal Husbandry and Dairying, 2022). One technology, artificial insemination (AI), has been instrumental in disseminating the best possible genetic resources in the form of semen, leading to enhanced milk production among the smallholder dairy farmers of India.

Due to growing mechanization in the agriculture sector, the demand for bulls as draught animals has been reduced drastically. Apart from this, male calves are lost from the system either through early calf mortality or are released into the village commons where they roam unattended. These released bulls become a nuisance for planned breeding interventions. Additionally, anti-slaughter laws in cattle hinder further progress in the dairy industry. The conventional AI technology alone will not be able to bring the desired improvement in building the dairy heifer stock. Therefore, a technology like sex-sorted semen (SSS), which can boost the production of heifers, is highly desirable for building a profitable smallholder dairy sector.

BAIF Development Research Foundation (https://baif.org.in/), located at Uruli Kanchan village near Pune, India, has been involved with dairy development programmes through door-to-door cattle and buffalo AI and other relevant services to about 2.65 million families of smallholder farmers in over 86 000 villages in 12 states by their 4 247 cattle development centres per annum. The present case study addresses the efficiency of use of SSS in the herds of Indian smallholder farmers.

Project approach

The performance of SSS has been successfully demonstrated in other countries. However, in India, at the time of implementation of the SSS pilot project in 2016, no such studies had been carried out to evaluate the performance of SSS under the smallholder dairy system. Therefore, no information was available to benchmark SSS performance in India.

The piloting of SSS was planned using the financial assistance of the Bill and Melinda Gates Foundation (BMGF) with a set objective to understand the performance of the technique in terms

Table 1. Cost of SSS inseminations to the farmers

Cost	No. of inseminations
Up to Rs. 100	85 598
Rs. 100 to 250	68 329
Rs. 250 to 600	48 402
Rs. 600 to 1 200	29 479
Rs. 1 250	23 585
Total	255 393

of conception rate and female birth ratio under diverse production systems. The piloting was also expected to provide guidance in framing appropriate policy decisions for the use of SSS.

The programme of SSS was carried out between November 2016 to December 2018 by importing SSS of pure Holstein Friesian and Jersey breeds. The initiative was implemented in nine Indian states, namely Uttarakhand, Uttar Pradesh, Bihar, Jharkhand, Odisha, Maharashtra, Andhra Pradesh, Telangana and Karnataka in over 700 cattle development centres. Training was given to all AI technicians for the usage, storage of semen and insemination. Simultaneously, the individual AI information, and its subsequent follow-up to calving, was stored using an online mobile application.

The unsubsidized cost of SSS was Rs. 1 250 (about 16 dollars) to the farmer. However, a few state governments and other stakeholders provided subsidies to introduce this technology at an affordable price. Sex-sorted semen was provided at the actual cost to farmers where the subsidies were not available (23 585 inseminations, just 9.2 percent of the total). The subsidized SSS prices resulted in comparatively higher inseminations as farmers had to pay less (231 808 inseminations, 90.8 percent of the total). Table 1 shows the prices paid by the farmers.

The SSS technology has a limitation of producing on average 90 percent female calves and 10 percent male calves. Inseminations were only carried out after obtaining consent from the farmer about the probability of this sex ratio. The consent is obtained from farmers to inform them about the possibility of producing 10 percent male calves. This helped to avoid future conflicts that might occur post-calving.

Technology of semen sexing

The SSS technology, developed and pioneered by the company Sexing Technologies based in the United States of America, was used in the semen sexing process. The technology is able to separate sperm bearing X or Y chromosomes on the basis of differences in their DNA content using a laser-based technology called flow cytometry. The semen is processed for sorting using the method described by González-Marín et al. (2018). The SSS production laboratory at the BAIF frozen semen station in Uruli Kanchan, Pune, is one of the best in India in terms of infrastructure, diverse bovine breeds availability and quality of SSS.

Every single batch of SSS produced was subjected to a quality standard protocol. Some of the standards included gender purity of more than 90 percent, the removal of dead sperm and ensuring a final concentration of at least 2.1 million sperms/straw.

Project results and its perceived impacts

A project of SSS use in farmers' herds was carried out in two phases. First was the piloting of imported semen of Holstein Friesian and Jersey pure breeds from Sexing Technologies. In this phase, 40 000 inseminations were carried out from November 2016 to December 2018 with a conception rate of 45.9 percent confirmed by transrectal palpation of pregnancy diagnosis 60–90 days after insemination on follow-up basis. Out of 13 113 calves produced, 11 814 (90.1 percent) female calves were produced. Contrary to the global recommendation of the use of SSS on heifers for better results (Borchersen and Peacock, 2009; DeJarnette, Nebel and Marshall, 2009; Frijters *et al.*, 2009 and De Vries, 2010), the success rates in multiparous animals were equally good.

The success rate in terms of conception and sex ratio under the smallholder dairy system led to BAIF initiating the second phase by setting up a SSS facility at the BAIF semen station to produce SSS of crossbreds, local cattle and buffalo breeds in November 2018. The conception rate of 43.3 percent with 90 percent female calves born out of 14 734 calvings was the outcome of 215 393 sorted semen produced at the BAIF facility.

Theoretical estimation of the economic benefits

Table 2 contains the results of an economic study comparing the use of SSS versus conventional non-sexed semen. In theory, this biotechnological intervention in the BAIF programme alone can result in the creation of an estimated asset worth 1 043 million rupees (about USD 13 million dollars) in the form of additional females produced and the potential to generate 2 085 million rupees (about USD 26 million dollars) worth of milk per year at the doorstep of smallholder dairy farmers from the third year onwards for the next six years or so, compared to the use of conventional semen.

In total, the estimated surplus worth produced by smallholder dairy farmers can be 3 128 million rupees (about 39 million dollars). However, these are not actual economic evaluations and may vary with the actual economic gain. Also, the study does not account for the additional costs of using SSS versus conventional semen. Similarly, actual economic analysis is not carried out of the impacts for the farmers of using SSS versus using conventional AI.

Table 2. Theoretical estimation of the economic value of heifers produced from conventional semen versus SSS

Parameters	Conventional semen	Sorted Semen
Total number of inseminations in 5 years	255 393	255 393
Conception rate (%) on actual basis	45.00%	43.90%
Estimated number of pregnancies	114 927	112 118
Female calf births	50%	90%
Number of female calves born	57 463	100 906
Female calf loss up to heifer stage	20%	20%
Mature heifers produced	45 971	80 725
Assumed market value of one heifer (rupees)	30 000	30 000
Total value of heifers produced (million rupees)	1 379	2 422
Surplus heifer value through use of SSS (million rupees)		1 043
Estimated average milk yield per lactation (kg)	2 000	2 000
Average milk price per litre (rupees)	30	30
Estimated value of milk produced from additional heifers produced by SSS (million rupees)		2 085
Estimated surplus net worth generated through SSS inseminations (million rupees)		3 128

Challenges encountered during the implementation process

Cost of SSS artificial insemination delivery

The major hurdle in the implementation process was the cost of single SSS insemination. In a developing country like India where conventional insemination costs range from Rs. 40 to 200 (0.5 to 2.5 dollars) the cost of SSS was almost six times higher, and therefore expensive for many smallholder farmers, despite its merit over conventional AI. As the technology is patented, it will be difficult to reduce the cost of production in the present scenario unless some new technology is developed with similar results under field conditions and at a lower cost. To solve this issue, BAIF approached different state governments, milk unions and companies to provide support to SSS services at a subsidized rate so that farmers would adopt this technology and could benefit from it. The financial assistance provided by different agencies helped to demonstrate the usefulness and performance of SSS to smallholder dairy farmers.

Birth of male calves

As per our results, 10 percent of male calf births were recorded. The farmers whose animals had male calves produced in their farm were nevertheless encouraged by the success stories of their neighbouring farmers. Because of this, 15 percent of these farmers opted for SSS for the next inseminations.

Mr Bhanu Mahato, a farmer from the Patratu village of Jharkhand State had 26 Holstein Friesian crossbred animals and had been using service from BAIF AI technicians since 2009. During an interaction with the farmer on his experiences with the use of SSS he replied, "I don't know whether the technology works or not but if I am able to get one less male calf, it will be a win-win situation for me, and the amount spent on SSS inseminations will be recovered." Nine of his animals were inseminated with SSS, out of which seven animals became pregnant. From these seven pregnancies, the farmer obtained seven female calves.

Figure 1. Interaction with an early adopter of SSS technology

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Disturbance in activities due to COVID-19 pandemic

The progress of SSS inseminations was halted by the emergence of the COVID-19 pandemic. The pandemic led to a complete or partial lockdown in the country, which reduced animal breeding activities. This reduced the number of inseminations and their subsequent follow-up compared to before the pandemic. Conditions got worse with the reduction in the price of milk, and farmers were forced to sell animals to meet urgent family needs. However, as the pandemic slowly subsided, breeding activities recovered and have now normalized.

Factors that contributed to the success of SSS implementation

Farmer awareness programmes

Awareness about the technology paved the way for its adoption by smallholder farmers. The farmer awareness involved mainly individual village-level farmer meetings, posters and local newspapers. To spread the benefits of SSS to a wider range of farmers, a mobile call tune (farmers get to hear this voice message when they call the AI technician to inseminate their cows) explaining the benefits of using SSS was prepared in local languages and was set on each AI technician's incoming calls so that farmers would get to know about the merits of the technology.

BAIF launched another initiative through their own call centres known as "samvadini", operated by female staff, where the operator calls the farmers to create awareness about the use of SSS, resolving any technical questions, doubts or misconceptions regarding the SSS or its use in Indian cattle and buffalo populations through expert opinion. Social media platforms like WhatsApp groups for farmers were also created and any news related to awareness programmes and success stories was distributed regularly.

Figure 2. Farmer awareness activities

(A) Farmer awareness programme: AI technicians' rally

(B) Project official disseminating the benefits of SSS

(C) Village-level farmer meeting

(D) Samvadini: Outbound call centre to create awareness about the use of SSS

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Figure 3. A successful farmer with crossbred progeny produced from SSS inseminations

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Use of SSS for high genetic merit bulls

The technology was intended to produce more heifers with high genetic potential. For this purpose, imported high genetic merit Holstein and Jersey bulls for production and reproduction characteristics were selected in phase I of the project on the basis of their phenotypic records and genomic estimated breeding values which indicate the genetic value of the animals for the respective production and reproduction characteristics. Later on, high genetic merit bulls of crossbreds, local cattle and buffalo breeds from the BAIF bull stud farm were selected on the basis of the dam's 305-days lactation yield for SSS production in phase II, resulting in males that are much more adapted to local production systems.

Experienced AI technicians

At the time of implementation, the technology was new for the farmers and its adoption was dependent on its success in smallholder dairy farms. To win the confidence of the farmers, implementation of the project was initiated through experienced AI technicians who had been working with the farmers experienced in conventional inseminations and who had achieved good conception rates. The AI technicians performed inseminations only when the cow/buffalo had shown proper heat symptoms and ensured the per rectum examination of the animals. This factor proved to be very successful as farmers who were hesitant to use SSS earlier slowly adopted the technology.

Figure 4. Verification of SSS doses by the programme officials at the farm

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Visible results in the ratio of female to male calves and conception rate in herds

A major factor for the success of SSS implementation in farmers' herds is the 90 percent birth of high genetic merit female calves compared with the outcome of conventional semen. This led to word-of-mouth publicity among the farmer community and resulted in increased demand for SSS. This, coupled with the subsidy provided by milk cooperatives, corporates and state departments, led to more adoption. The birth of more females also resulted in fewer calving problems, thereby improving the future conception rate. Out of 27 831 calvings, only 70 (0.25 percent) were difficult. This indicated a lower incidence of dystocia in animals inseminated with SSS in the programme. In addition, problems related to male calf disposal declined, resulting in decreased economic loss. The surplus female calves born also served as an income source, as they could be sold as surplus heifers.

Access to sorted semen

The infrastructure for the SSS delivery system was built to ensure an uninterrupted supply of good quality semen with doorstep delivery to the farmers' herds. The SSS was dispatched to AI technicians once or twice a month with a regular supply of liquid nitrogen.

Real-time tracking of the SSS straws

A mobile based software solution was developed to track semen supply, semen repositories at individual AI technicians, delivery and record keeping of individual inseminations and pregnancy diagnosis up until the birth of the newborn calf. Straws used for insemination were kept by the farmer with a signed consent copy.

Trust of farmers in BAIF

BAIF has been working with farmers for more than 50 years to improve their livelihoods through livestock and other related activities. The uninterrupted quality service and support for the dairy development programmes offered by BAIF is instrumental in building trust between farmers and BAIF, which helped BAIF introduce SSS under the Indian smallholder system despite the higher price.

Subsidies to make SSS affordable to smallholder farmers

With the high financial burden of individual SSS inseminations for farmers, it was necessary to try to reduce the cost. The main aim of the programme is to encourage farmers to adopt the newly proven technology that could help them to increase their herd size, create replacements for their older cows, prevent the birth of unwanted male calves (due to anti-slaughter laws in the country) and subsequently increase milk production, thereby boosting the income of smallholder dairy farmers. The government wants farmers to adopt the SSS technology because many farmers release their male animals, which then become a nuisance in terms of damage and loss of agricultural crops from uncontrolled grazing, disturbances to vehicles, difficult-to-control contagious diseases and, crucially, indiscriminate breeding leading to genetic erosion.

The SSS initiative was supported by subsidies from different state governments and other agencies working closely with the dairy and livestock sectors. The cost of inseminations to farmers, most of which were subsidized, is presented in Table 1. Subsidies to reduce the cost of SSS inseminations were provided by state governments like Uttar Pradesh, Jharkhand, Odisha, government agencies like the National Bank for Agriculture and Rural Development (NABARD) and dairy cooperatives like Godavari Dairy Private Limited. Apart from governments, subsidies were also provided by different companies under their corporate social responsibility activities to promote the use of SSS among smallholder farmers. All of these are once-off subsidies and hence not available every year.

Lessons learned from the implementation of SSS in smallholder farms

Selection of animals for SSS AI

Considering limitations such as the cost of SSS delivery to the farmer and low sperm concentration (approximately 2 million sperm per dose), it was important to select animals with a normal estrous cycle, have proper heat detection, measure body weight as an indirect way of confirming physiological development and to use cows with no history of repeat breeding, preferably heifers. The selection of animals with these criteria increased the likelihood of animals becoming pregnant, and farmers incurred fewer economic losses.

Successful use of SSS in multiparous animals

According to the literature, it is usually advised to use SSS in heifers only (Borchersen and Peacock, 2009; DeJarnette, Nebel and Marshall, 2009; Frijters *et al.*, 2009). In the pilot study, SSS was used not only in heifers but also in multiparous animals, with good results both in terms of conception rate and the birth of female calves.

Farmer consent

It is highly recommended to obtain consent from the farmers about the possibility of the offspring being 10 percent male before performing insemination with SSS.

Conclusion

BAIF will continue to explore the possibility of providing smallholder dairy farmers with access to valuable and high genetic merit bulls' SSS at an affordable cost, to improve genetic progress by cross-subsidizing SSS delivery cost through milk unions, state governments and private companies under corporate social responsibilities. Specific efforts can also be undertaken for local breeds that are on the verge of becoming extinct. Through a special breed development programme, with the use of SSS for faster multiplication of the female population, the respective breed can increase its population size within a stipulated time. In addition to this, the use of SSS coupled with genomic selection in cattle and buffaloes will help to increase the rate of genetic gain of the endangered breeds, thus making them more economically competitive and more sustainable in the long term.

The scope will also expand to produce Y-sorted semen in the future for some of the draught purpose cattle breeds like Khillar, Hallikar and others. Apart from these initiatives, BAIF is looking forward to producing high genetic merit embryos using SSS to multiply the genetic pool as well.

Acknowledgments

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2.3 Applying genomics to identify optimal crossbred cattle genotypes for sustainable smallholder dairy production in Bangladesh

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Background and context

Bangladesh is primarily an agriculture-based economy, responsible for around 14 percent of the national gross domestic production. More than 40 percent of Bangladesh's labour force is involved in agriculture. Dairy farming is an important component of Bangladeshi agriculture with the involvement of 3.1 million households in cattle rearing (Bangladesh Bureau of Statistics, 2019). During the last ten years, annual milk production in Bangladesh increased from 5.1 million tonnes in 2012–13 to 13.1 million tonnes in 2021–22 with an annual growth rate of 15.8 percent (Department of Livestock Services, 2022). There are about 24.7 million cattle, of which around 4.1 million are milking cows (Department of Livestock Services, 2022) that contribute about 90 percent of milk produced in the country. The remaining milk comes from goats (about 8 percent) and buffalo (2 percent) (Datta, Haider and Ghosh, 2019).

Dairy cattle production in Bangladesh is predominantly characterized by a smallholder farming system with about 1–5 animals per household. For example, in the Sakthira district of southwest Bangladesh about 45 percent of dairy farms have 1–3 animals, 34 percent have 4–5 animals and 21 percent have more than 5 animals (Datta, Haider and Ghosh, 2019). These smallholder dairy production systems contribute to increased household incomes and the accelerated growth of the rural economy, and thereby improved food and nutritional security.

The native cattle of Bangladesh are zebu type (*Bos indicus*, with a hump) that are developed mainly for draught purposes. Their milk productivity is low, with an average of 1–2 kg/day. The native cattle of Bangladesh are represented by non-descript desi, Pabna, Red Chittagong and North Bengal Grey cattle. They are well adapted to the harsh tropical climate and have the ability to produce with minimal feed resources, like crop residues. On the other hand, the commercial dairy cattle developed for high milk productivity are *Bos taurus* type (without a hump) and can produce 30–40 kg/day.

The vast majority of smallholder dairy farmers in Bangladesh possess zebu cattle and use low external inputs (concentrates, green fodder, housing etc.) for cattle production. Most of these farms follow conventional practices on feeding, breeding and disease management. The reliable and sustainable supply of improved animal genetics is one of the major impediments for increased productivity in these smallholder systems.

Hand milking of a crossbred cow by an animal husbandry worker in September 2022 at Phulpur, Mymensingh

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Genetic improvement of dairy cattle in Bangladesh is attempted through crossbreeding, using semen from commercial taurine breeds like Holstein-Friesian and Jersey. In such programmes, the genetic make-up of crossbred cattle needs to be stabilized to select and develop the optimal genotype(s) that can perform efficiently under the existing production system. However, such stabilization of crossbreds did not materialize at the field level due to a lack of organized breeding infrastructure (e.g. pedigree and performance recording), technology constraints in establishing the genetic make-up of crossbreds in the absence of pedigree records and the tendency among farmers to increase taurine blood as much as possible with the expectation of increasing the productivity per animal. This has resulted in indiscriminate crossbreeding, leading to problems of adaptability, fertility and susceptibility to heat, humidity and tropical diseases.

Establishing a pilot performance recording system

In 2016, the Bangladesh Agricultural University initiated a programme on applying genomic and reproductive biotechnologies for the genetic improvement of crossbred cattle in collaboration with the Central Cattle Breeding Station (CCBS) of Bangladesh. The aim was to establish a pilot performance recording system among small- and medium-sized dairy farms, and apply advanced genomic information at the field level to determine the genetic make-up of crossbred dairy cattle. The project was supported by the Animal Production and Health Section of the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture and the technical cooperation program of the International Atomic Energy Agency (IAEA).

A well-ventilated shed of crossbred cows at Phulpur, Mymensingh

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A total of 11 upazilas (sub-districts) located across four different administrative divisions in different regions of Bangladesh (Dhaka, Chattogram, Mymensingh and Rajshahi) were identified for implementation of the project. More than 60 private and 2 public dairy farms were selected and registered for performance recording. About 30 percent of the farms registered for the study were small, with a herd size of five or less animals, while the size of remaining farms ranged from 6 to 75 with an average of 35. A farm data register was supplied to each of these farms and information on various management practices, including housing, feeding, breeding and milking frequency, was recorded for each registered farm. About 1500 cattle were ear tagged and identified for establishing a pilot performance recording system.

The dairy cattle farms were randomly selected for performance data recording from each of the four regions. Management practices in the selected farms were typical of their respective regions except for minor differences in terms of quantity of concentrates provided to cows, the availability of green fodder, the affordability of farmers to provide optimal housing and feeding, etc. Such information was collected at the beginning of performance data recording and was subsequently considered in the statistical model while analysing the phenotypic data.

All the registered cows were crossbreds of Bangladeshi local zebu and commercial Holsteins with varying levels of exotic blood. Most dairy farms in Bangladesh maintain that crossbred cattle and the use of local zebu cattle for milk is very limited, except for home consumption. Some of the farms registered for the study included a few local zebu cattle, but they were not included in the performance recording scheme because crossbreds rather than zebu cattle were the targets.

In the farm, records were made of the phenotypic and performance data of cows, including the date of calving, parity, test day milk yield, date of service, date of drying, number of inseminations

per successful conception, age at first service and age at calving. A participatory approach was followed by involving farmers in the process of data recording. The project team visited the farms at 1–2-month intervals and collected the data using questionnaires, farmer interviews and farm records. The data were digitized regularly at the Bangladesh Agricultural University resulting in the generation of more than 4 000 records at the field level. Apart from phenotypic data collection, blood samples were collected from each of the registered cows and DNA was extracted for genome analysis. As a first step towards establishing a reference population to implementing a genomic selection programme, the Bangladesh Agricultural University set up a national biobank of DNA samples from performance recorded cattle. A subset of the DNA samples was transferred to the Joint FAO/IAEA laboratories in Austria for genome-wide typing.

Biotechnology intervention

In general, exotic blood levels in crossbred cattle can be estimated using pedigree records maintained in the dairy farm. In smallholder dairy production systems, maintaining such records is extremely difficult due to several challenges, including a lack of information on semen used for insemination, the purchase of animals without records from local markets and a lack of awareness among farmers. Molecular/genomic technologies can help to overcome this challenge and estimate the genetic composition of crossbred cattle with high accuracy. DNA markers, like short tandem repeats (STRs) or single nucleotide polymorphisms (SNPs) can help in assessing the level of genetic admixtures in populations. With advances in genomic technologies, it is now possible to type individual cows for thousands of genome-wide markers at a relatively low and affordable cost.

Briefly, the state-of-the-art DNA microarray technology (DNA chips containing about 60 000 markers) was used at the Joint FAO/IAEA Centre in Vienna to establish the genetic make-up of the crossbred cattle. A database on genome-wide SNP genotypes was established for purebred Holstein cattle and for local Bangladeshi zebu cattle.

Bangladeshi zebu cattle are local non-descript cattle and no pedigrees are maintained. With few exceptions, most samples of purebred zebu cattle were collected from farms that were not part of the group of 62 farms registered for performance recording. In the absence of pedigree records, the following criteria were used to ascertain the purity of sampled purebred zebu cattle and use them as the reference population:
- morphological features (presence of hump, coat colour etc.)
- breeding history based on farmers' interviews
- estimated taurine ancestry based on STR and SNP genotypes (zebu cattle with >1 percent taurine ancestry were excluded from the reference population)

With respect to the reference population of purebred Holstein cattle, samples from different countries were utilized (Bangladesh, India, Serbia and Sri Lanka) where pedigree information was available from institutional/commercial farms.

The genome-wide SNP genotypes of crossbred cattle were analyzed alongside the SNP genotypes of purebred reference populations to estimate genetic admixture and establish the level of taurine blood in them. Each crossbred cow was assigned to one of six genotype groups: >87.5 percent taurine, 75–87.5 percent taurine, 62.5–75 percent taurine, 50–62.5 percent taurine, 25–50 percent taurine and <25 percent taurine.

**Figure 1. Genetic composition of crossbred cattle in smallholder dairy farms
of Bangladesh**
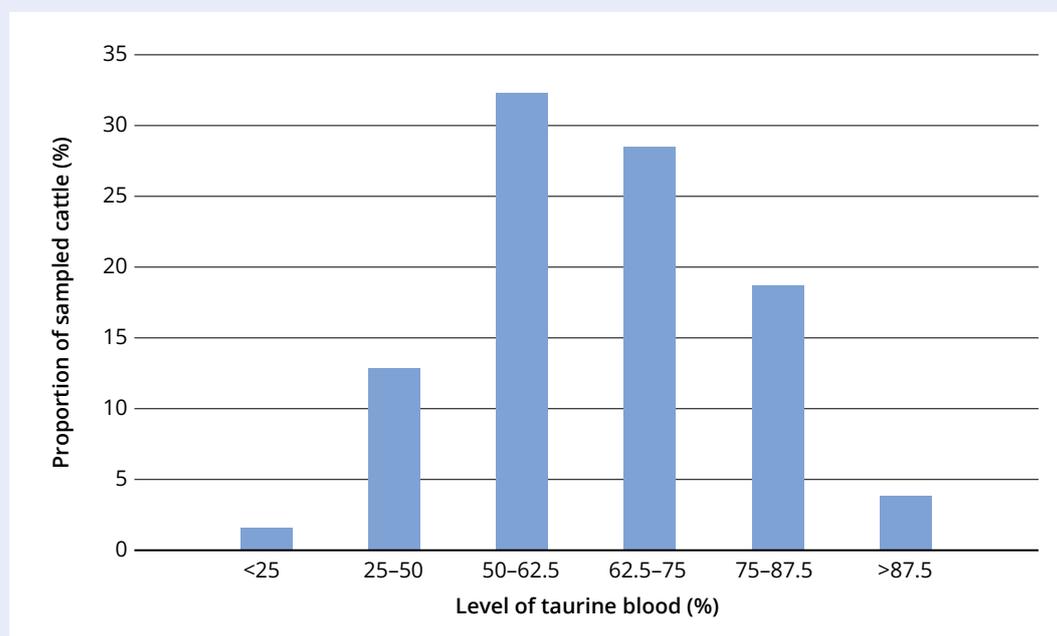
Figure 1 shows that the 50–62.5 percent taurine genotype group was the most frequent among the Bangladeshi crossbred cows, followed by the 62.5–75 percent and 75–87.5 percent taurine genotype groups. The proportion of different crossbred genotype groups in each of the four major regions was examined. There were regional variations, with a higher proportion of 50–62.5 percent taurine genotypes in the Dhaka region while 62.5–75 percent taurine genotypes were most frequent in the Chattogram and Mymensingh regions. In the Rajshahi region, more than one-fifth of the crossbred cows had less than 50 percent taurine genetics. The relatively low level of taurine genetics in the Rajshahi region indicates that farmers were (i) backcrossing their crossbred cattle with local zebu bulls due to productivity/adaptability issues or inability to provide an optimal balanced diet (concentrates, green roughages, etc.) needed by high producing cows; and/or (ii) unable to get timely access to artificial insemination services and hence resorting to natural service with zebu bulls available locally.

Progress to date

Evaluation of the production performance of Bangladeshi crossbred cattle revealed that, up to levels of 75 percent taurine blood, milk yield increased from the first to the third lactation and started declining gradually in subsequent parities (Figure 2). In all lactations, the estimated milk yield rose with increasing taurine blood from 25–50 percent (1 522 to 2 278 litres) and up to 62.5–75 percent (1 724 to 2 798 litres) in crossbred cows. The milk yield started decreasing with further increase of taurine blood in crossbred cattle.

Figure 2. Estimated milk yield of crossbred cows with different levels of taurine blood under smallholder dairy production system
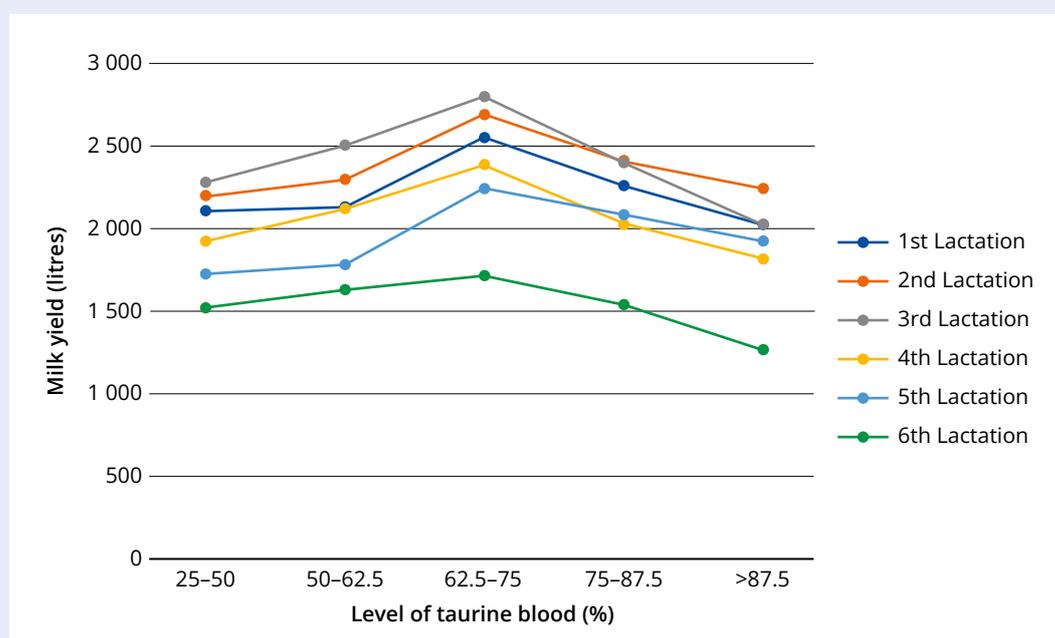
With respect to reproductive performance, the number of services per conception was lowest for 50–62.5 percent and 62.5–75 percent taurine groups and it increased when the level of taurine blood increased beyond 75 percent. Similarly, first service conception rate was highest in these two groups (50–62.5 percent and 62.5–75 percent taurine blood) while it started declining in cows with more than 75 percent taurine blood.

Table 1. Reproductive performance of crossbred cattle with different levels of taurine inheritance

Taurine genetics %	Age at first service (days)*	Cows inseminated	Cows conceived at first service	First service conception rate*	No. services/ conception
25–50%	516.1±81.6[ab]	312	130	41.7[ab]	1.9
50–62.5%	560.8±84.1[a]	815	385	47.2[b]	1.7
62.5–75%	519.0±56.0[ab]	729	345	47.3[b]	1.7
75–87.5%	485.3±49.1[b]	506	210	41.5[ab]	1.9
>87.5%	501.1±37.5[ab]	98	33	33.7[a]	2.1

* Means with different superscripts within a column differ significantly (P<0.05)

An animal husbandry worker carrying cultivated green fodder for crossbred cows
at Phulpur, Mymensingh

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In general, the productivity of crossbred cattle (lactation milk yield) increases with increasing levels of taurine blood in crossbred cows, but such genotypes need to be supported with an optimal production environment, in terms of providing optimal dietary requirements (like adequate concentrates, green roughage and mineral supplements), housing and veterinary care (Mujibi *et al.*, 2019; Getahun 2022). The small dairy farmers in Bangladesh mostly rely on crop residues and communal grazing and often have financial limitations in affording adequate levels of concentrates to feed their animals. Further, certain levels of zebu genetics need to be retained in the crossbreeds to preserve the adaptability characteristics related to tropical heat, humidity and diseases.

The case study clearly demonstrated that the prevailing smallholder production system in Bangladesh cannot support crossbred cows when the taurine inheritance exceeds 75 percent. The level of taurine blood needs to be stabilized between 50 percent and 75 percent for optimal milk production and better reproductive efficiency of these dairy animals.

Impact and conclusion

With the application of genomics, information on the genetic make-up of about 1 500 crossbred cattle from over 60 farms has been established with a high level of accuracy despite the absence of pedigree records. Similarly, the genetic composition of breeding bulls used for frozen semen production in government bull stations has also been confirmed using genome-wide information.

Insemination of a crossbred cow with frozen semen at Phulpur, Mymensingh

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The smallholder farmers were provided with information regarding the genotypic status (taurine blood level) of their crossbred cows/heifers and technical advice was given on the right kind of semen (50 percent crossbred, 75 percent crossbred or purebred exotic) to be used for breeding. For example, in certain areas of Bangladesh where the farmers can afford to provide good quality feedstuffs, like concentrates and green fodder, advice was provided to have crossbred cows with up to 75 percent taurine blood level. In certain other rainfed areas where farmers rely predominantly on crop residues and communal grazing and cannot access/afford adequate concentrates throughout the year, advice was given to stabilize the taurine blood around 50–62.5 percent. Such information can help farmers to request and get appropriate bull semen straws for their cows/heifers from the artificial insemination (AI) service provider. The project team also exchanged the information with the government bull stations so they could produce and distribute the semen of sires with a suitable genetic make-up for AI in various regions of Bangladesh.

Application of genomics and AI, combined with data recording, has helped not only in providing customized breeding services to farmers, but also paved the way to stabilize and optimize the crossbred cattle population for sustainable improvement of milk productivity. Considering the long generation interval of cattle (4.5 to 5 years), the outcome in terms of stabilization of crossbred cattle (with desirable taurine blood level, optimal production performance and reduction in adaptability issues) is being quantified. The project team is also collecting information and samples to ascertain the genetic composition of offspring born after the genetic make-up of their parents was estimated using genomic information.

However, the genotyping service will not be a routine practice for every new animal born in the registered farms as it will not be economically sustainable. Initially, the genotyping data will be utilized to provide information on the genetic make-up of crossbred cattle to the farmers so that they can make decisions regarding the appropriate bull genotype to be used for AI. Once the existing breedable females (and breeding bulls) are genotyped and genetic composition estimated, the breeders can use this information to predict the genetic composition of newborn animals. Genotyping will continue for animals in newly registered farms for performance recording. Over time, this approach will pave the way for establishing a reference population with pedigree and performance data for implementing a genomic selection programme to improve crossbred dairy cattle in the country. The national biobank of DNA samples from performance recorded animals established under the project will form the basis for a reference (training) population required to set up a genomic prediction machinery and estimate the breeding value of bulls used in the ongoing artificial insemination programme. Such a breeding programme based on genomic information will not only result in better productivity but will also stabilize the crossbred cattle with optimal taurine admixture compatible to the local production system environment.

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2.4 Use of filter paper to rapidly diagnose African swine fever in Madagascar

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Background

The world's population is currently growing, but stocks of vital natural resources such as animal genetic resources, agricultural land and water are gradually diminishing. To feed the world in this situation, the development of short-cycle livestock, especially pork, would be a plausible approach. It has a positive impact on a large part of the population through its value chain which involves several types of actors including producers, live pig traders, pork processors, consumers and many other actors who use pork and its by-products for traditional, cultural, religious and medicinal purposes.

African swine fever (ASF) is a highly contagious viral disease, caused by a virus belonging to the family Asfarviridae and genus Asfivirus (Dixon *et al.*, eds., 2005). It is transmissible, affects wild and domestic swine and has very severe epidemic characteristics with very high mortality rates (Atuhairwe *et al.*, 2013). Worldwide, ASF remains a major health threat and limits the development of pig farming, compromising the food security of many countries (Rasamoelina-Andriamanivo, Porphyre and Jambou, 2013).

In Madagascar, this disease was unknown until it had the first diagnosis in December 1998. Thereafter, it spread over almost all the country (Humbert, 2006). ASF caused numerous economic losses and major disruptions in the pig industry, as well as the disappearance of many improved livestock farms (Franco, 2007; Grangé, 2016).

There is still no effective treatment or vaccine against ASF, so the disease can only be controlled by preventive sanitary measures. The lack of medical prophylaxis and treatment of ASF requires rapid diagnosis in order to control and eradicate the disease (Randriamparany *et al.*, 2016). In tropical countries, however, the diagnosis of human or animal viral infections is often hampered by the need to maintain a cold chain for the preservation of samples to the laboratory. The cold chain is necessary to transport infectious material for initial or confirmatory diagnosis of ASF infection

to local, regional or international laboratories. In remote areas, especially in developing countries without adequate infrastructure, it is often impossible to maintain a cold chain for the storage of biological samples. There is therefore an urgent need for a method of sampling and transport from farm to laboratory that does not rely on the cold chain.

In recent years, studies have demonstrated that samples could be poured and stored on filter papers at room temperature for relatively long periods (Uttenthal *et al.*, 2013). The use of blood samples dried on filter papers was described as a possible alternative to preserving human and animal samples for testing (Abdelwhab *et al.*, 2011; Joseph and Melrose, 2010).

Figure 1. Room temperature storage of filter papers

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The diagnosis of ASF is based on virus or antibody detection. Many techniques can be used but the choice of a test depends on financial means, materials and techniques, the level of urgency and the virus strain in question (Franco, 2007). Whatman 3MM filter paper was used to collect pig blood (Figure 1) in this study, because it is difficult to send conventional samples from the field to the laboratory due to the cold chain requirement. The same sample was used to detect both the ASF virus, using the polymerase chain reaction (PCR) technique, and the presence of circulating antibodies during viral infection by the enzyme-linked immunosorbent assay (ELISA) technique.

PCR is a laboratory technique for rapidly producing and amplifying millions to billions of copies of a specific segment of DNA, which can be studied in greater detail. ELISA is used for detecting the presence of an antigen in biological samples. An ELISA, like other types of immunoassays, relies on antibodies to detect a target antigen using highly specific antibody-antigen interactions.

Case study

This case study involves suspected outbreaks of ASF in different localities of Madagascar from late 2019 to early 2022. Investigations were carried out in seven different communes during outbreaks (Figure 2). During the outbreaks, samples were collected from the sick pigs still on the infected farms and also from the apparently non-infected surrounding farms. A total of 198 filter paper samples impregnated with pig blood were collected, and analysis of the samples was carried out at the Laboratoire National de Diagnostic Vétérinaire (LNDV, the National Laboratory of Veterinary Diagnosis), located at Itaosy, Antananarivo.

The types of farms and husbandry practices involved a very low level of biosecurity and farms are not fenced. As previously indicated, Whatman 3MM filter papers, which are often used for storage and detection of genetic or protein material, were selected for this study (Randriamparany *et al.*, 2016). The filter papers were cut into 5 x 0.5 cm strips and then impregnated with blood from slaughtered pigs and farms after making a small incision in the throat, then dried and stored in envelopes at room temperature until use. Once dried, samples can be sent, such as to an analysis

laboratory, quickly and easily. This technique can be used in surveillance or vigilance studies and allows early detection of virus circulation, and thus allows farmers to take control and protection measures for their animals in a timely manner.

Diagnosis procedures

Conventional direct PCR

The highly conserved region of the genome encoding the p72 protein of the ASF virus was amplified by PCR using the five prime Mastermix (Eppendorf, Montesson, France). Pieces of 3MM filter papers with dried blood from infected pigs were placed directly into PCR tubes without prior nucleic acid extraction. A 2 mm^2 piece of filter paper was placed in each 0.2 ml PCR tube. The reaction mixture was added to a final volume of 50 µl to allow for proper immersion of the filter papers. The reaction mixture contained 0.4 µM of direct primer: 5'-T CGGAGATGTTCCAGGTAGG-3' and reverse primer: 5'-GCAAAAGGATTTGGTGAAT-3'. The PCR was run as follows: (i) 5 min at 95°C; (ii) 35 cycles for 30 s at 95°C, 30 s at 55°C and 30 s at 72°C; (iii) 7 min at 72°C. A PCR fragment of 346 base pairs was visualized on an agarose gel. A negative control from an uninfected pig was included. Fragment size was defined by comparison with DNA ladders.

Antibody detection by ELISA

A 40 mm^2 piece of 3MM filter paper containing dried blood was collected and added to a 100µl volume of ELISA buffer (Ingezim PPA Compac, Ingenasa, Spain). After incubation for two hours, the eluate was collected and tested with a designed ELISA kit.

Results

Using 3MM blotting paper for the samples, the detection of the ASF virus by direct PCR gave positive results in 39 of the 198 samples (an overall 19.7 percent prevalence rate). No positive results were observed for the detection of ASF antibodies. The results are summarized in Table 1.

Table 1. Detection of the ASF virus by the PCR technique using 3MM filter paper samples impregnated with pig blood, according to year and location

Year	Region	District	Commune	3MM filter papers		Pig numbers in the municipality
				Collected	Positive	
2019	Diana	Ambanja	Ambanja	1	1	9 500
		Ambilobe	Ambilobe	68	7	24 000
2020	Menabe	Belo	Belo	7	3	4 000
		Morondava	Morondava	20	1	6 000
2021	Itasy	Arivonimamo	Imeritsiatosika	14	9	22 000
	Sofia	Port Bergé	Port Bergé	26	14	600
	Fitovinany	Vangaindrano	Vangaindrano	25	3	400
2022	Menabe	Morondava	Morondava	37	1	6 000
Total				**198**	**39**	**72 500**

Figure 2. Map of Madagascar with the locations of African swine fever virus outbreaks
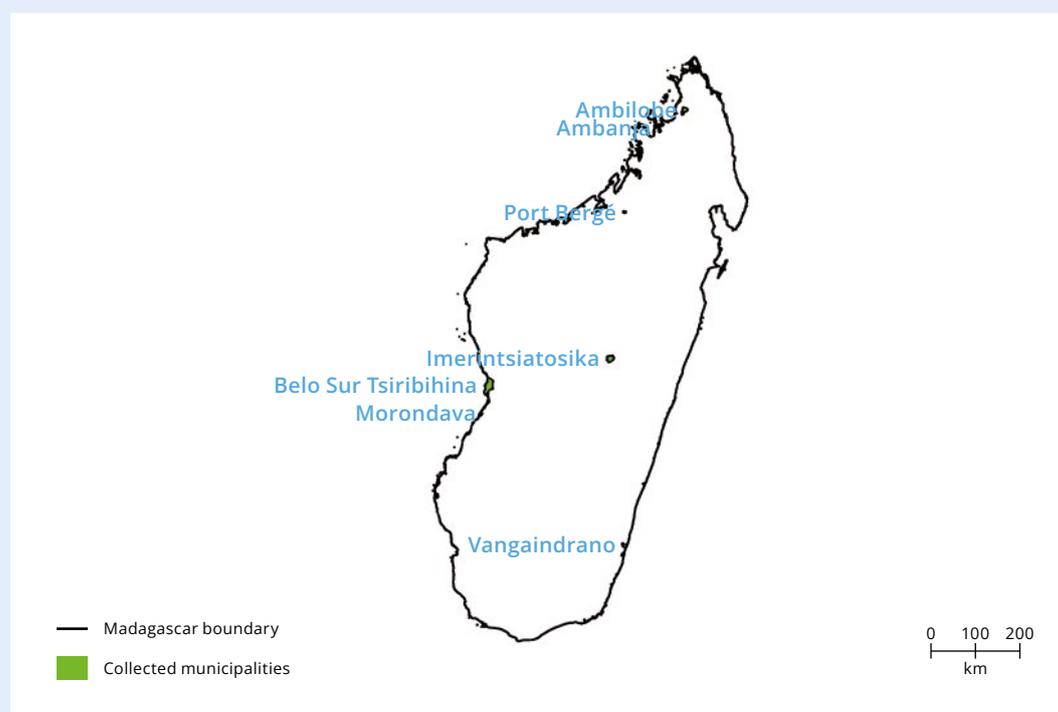
Discussion

This technique was successfully validated with experimentally infected pig blood (Michaud *et al.*, 2007). Another validation of this technique was performed by Randriamparany *et al.* (2016) by comparing filter papers with standard field samples.

In this case study, we did not find any positive samples for antibodies when testing 3MM blotting papers using the ELISA kit. This result is consistent with the circumstances by which antibodies were not yet circulating in the animals' blood at the time of sampling. Antibodies are usually detected around the 14th day of virus incubation (Gallardo, Fernández-Pinero and Arias, 2019). Another possible explanation is that low concentrations of antibodies may be less detectable in 3MM filter papers compared to sera. On the other hand, a prevalence of 19.7 percent (95 percent CI: 14.4–25.6 percent) was found using molecular techniques (PCR) which are the most popular diagnostic procedures used for rapid identification of animal and human diseases.

Based on these results, this study suggests that the Whatman 3MM filter papers can be used for ASF diagnosis using a series of currently available tests that were originally designed for the detection of ASF virus and antibodies in conventional biological samples. No direct comparison with conventional samples was done, however, in this study.

Whatman 3MM filter papers do not contain additives. Therefore, they can preserve infectivity and can theoretically be used for further amplification of pathogens. Another advantage is that they do not contain PCR inhibitors and can be used directly in conventional PCR without prior nucleic acid extraction, as previously demonstrated in the detection of ASF virus (Michaud *et al.*, 2007; Randriamparany *et al.*, 2016). This technique is quick, easy to perform and does not use cold chains which usually cause problems in most developing countries.

In this study, the performance of Whatman 3MM filter papers for blood collection on blotting paper and storage at room temperature with current diagnostic procedures yielded convincing results for the detection of ASF. The observed specificity for this test on filter papers containing dried blood was excellent (100 percent) in a previous study (Randriamparany, *et al.*, 2016). It was also shown that 3MM filter papers have a remarkable advantage over conventional biological materials because nucleic acid extraction is not required. Thus, to be able to perform conventional direct PCR, there is a considerable reduction in the time needed for molecular diagnosis and cost. In addition, another potential benefit is the reduction of potential contamination during sample processing because samples are dried and prepared on small pieces of 3MM filter paper.

It was possible to use the Whatman 3MM filter paper technique for very early detection of infection by conventional direct PCR. Early detection is important because it has been reported that, in cases of ASF outbreaks, farmers in Madagascar rush to sell or slaughter their pigs to avoid economic losses (Randriamparany *et al.*, 2005). The same is true in other African countries where ASF is prevalent (Dione *et al*, 2014). In Madagascar, the government orders the slaughter of pigs on ASF-infected farms. Pigs on the farms where the 39 positive animals were detected were therefore slaughtered.

Conclusion

Whatman 3MM filter papers are an inexpensive, simple and fast way to collect blood, store samples and diagnose ASF disease by ELISA and conventional direct PCR. Advantages of 3MM filter paper strips include the smaller volume of blood required and the ability to collect a large number of samples in the field. Whatman 3MM blotting papers can be used as a multi-purpose medium for versatile diagnosis under tropical conditions.

African swine fever is a major health problem for the pig industry. One of the challenges of diagnosis, especially for African countries, is to be able to rapidly diagnose the presence of the virus on samples that are easy to collect and that do not require special equipment for storage. The test we used meets these requirements. It is a direct PCR test (conventional) on blood samples taken on blotting paper. After impregnation, the blotting paper is dried and a fragment of about 2 mm^2 is placed directly into a PCR tube, into which the reaction mixture is added. The use of this technique has shown promising results and has saved several farms in Madagascar since 2019.

In this case study, Whatman 3MM blotting paper proved to be a good support for the collection and storage of pig blood coming from a distant locality without using a cold chain to detect the presence of ASF in Madagascar. The method is currently being used by the LNDV to detect and analyze ASF outbreaks.

Acknowledgment

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2.5 Study of the genetic diversity of the Philippine Carabao swamp buffalo in the Philippines

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Abstract

The Philippine carabao (*B. bubalis* swamp type) is a source of meat, milk and draught power, and its manure is an excellent component of organic fertilizer. The population of swamp buffalo has always been considered as a single and homogenous breed in the country. Its characterization has usually been addressed through phenotypic information. However, due to the limitation of morphological-based identification systems, the local status of buffalo sub-populations raised the need to assess the between-breed diversity using a molecular approach. The overall concept of this project arises from a simple yet profound question: "Is there really genetic diversity among Philippine carabao sub-populations?" Answers to this question would direct the national conservation efforts for the Philippine carabao population. A total of six Philippine carabao sub-populations were analysed based on polymorphic microsatellites that can be used to understand the phylogenetic relationship and genetic diversity of swamp buffalo in the country. Results confirmed the presence of genetic diversity in the Philippine carabao sub-populations. Two sub-populations, on Calayan Island and Pitogo Island, emerged as distinct clusters and purebred swamp buffaloes. This implied the high affinity of these two swamp buffalo populations in their native tract. These are isolated islands for which travel can take 30 minutes (Talibon to Pitogo Island) to five hours (Aparri to Calayan Island) by boat from the mainland. The physical barrier caused by great bodies of water could impede genetic exchange between the neighbouring sub-populations, which most likely resulted in isolation of populations by distance. Aside from identifying these distinct swamp buffalo clusters, these sub-populations are known sources of pure-type swamp buffaloes. Strategic conservation and management of genetic diversity in the Philippine carabao should be prioritized.

Background

Water buffaloes (Bubalus bubalis) are distributed worldwide in the Indian subcontinent, China, Brazil, Italy and predominantly across southeast Asia (Lau *et al.*, 1998; Kierstein *et al.*, 2004; Cruz, 2012). In addition, these are economically important livestock species in many developing countries regarding their vital contribution to agriculture and the rural economy (Zhang *et al.,* 2007).

The buffalo species are divided into swamp and riverine, which differ in cytogenic and morphological characteristics. For example, the swamp buffalo has 48 chromosomes, while the riverine buffalo has 50. The typical physical features of swamp buffaloes are the white or light grey markings in the lower jaw and brisket (chevron) and the lighter skin and coat colour than the riverine buffaloes. In addition, the sickle-shaped horns of swamp buffalo extend backward compared to riverine buffaloes having heavily curled horns (Castillo, 1998).

Figure 1. A swamp buffalo dam and her calf grazing in Bohol province, Pitogo Island

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The Philippine carabao (*Bubalus bubalis*) swamp buffalo (Figure 1) are valuable agricultural livestock that are a primary source of draught power for farm cultivation. It is a secondary source of milk, hides and manure as a suitable substrate for organic fertilizer. However, formal sub-populations of the swamp buffalo are unrecognized and the species is considered a single and homogeneous breed in the Philippines (Paraguas *et al.*, 2018).

The overall aim of this study was to find if there is genetic diversity among Philippine carabao sub-populations. The results of the study could direct the national conservation efforts of swamp buffaloes to two possibilities: (1) No, genetic diversity does not exist. For example, suppose there is little to no genetic diversity among Philippine carabao sub-populations. In that case, the current institutional conservation programme for Philippine carabao at Cagayan State University will be enough, or (2) Yes, genetic diversity is present. If there is genetic diversity among Philippine carabao sub-populations, this calls for separate strategic conservation and management activities for the distinct sub-populations. Thus, it was pertinent to carry out a comprehensive study on the genetic diversity of the Philippine Carabao using genetic markers, such as microsatellites. Microsatellites are short sequences of DNA (usually from one to six base pairs in length), that are repeated multiple times in a given genomic location. Genetic variation is based upon the number of repeats.

The conservation and management of swamp buffaloes in an open nucleus herd, village-based or institutional herds have a common practice of selecting animals based on size, growth rate, reproduction ability and phenotypic information (Cruz, 2012; Paraguas *et al.*, 2018). However, morphological-based identification systems could be limited when identifying swamp buffaloes because morphological variation could be greater within sub-populations than among them. A more informative option may be to use genetic markers to assess the genetic diversity and population structure of swamp buffaloes in the country.

Figure 2. Map of the Philippines showing the different blood sample collection sites on the three main island groups of the Philippines, namely Luzon, Visayas and Mindanao
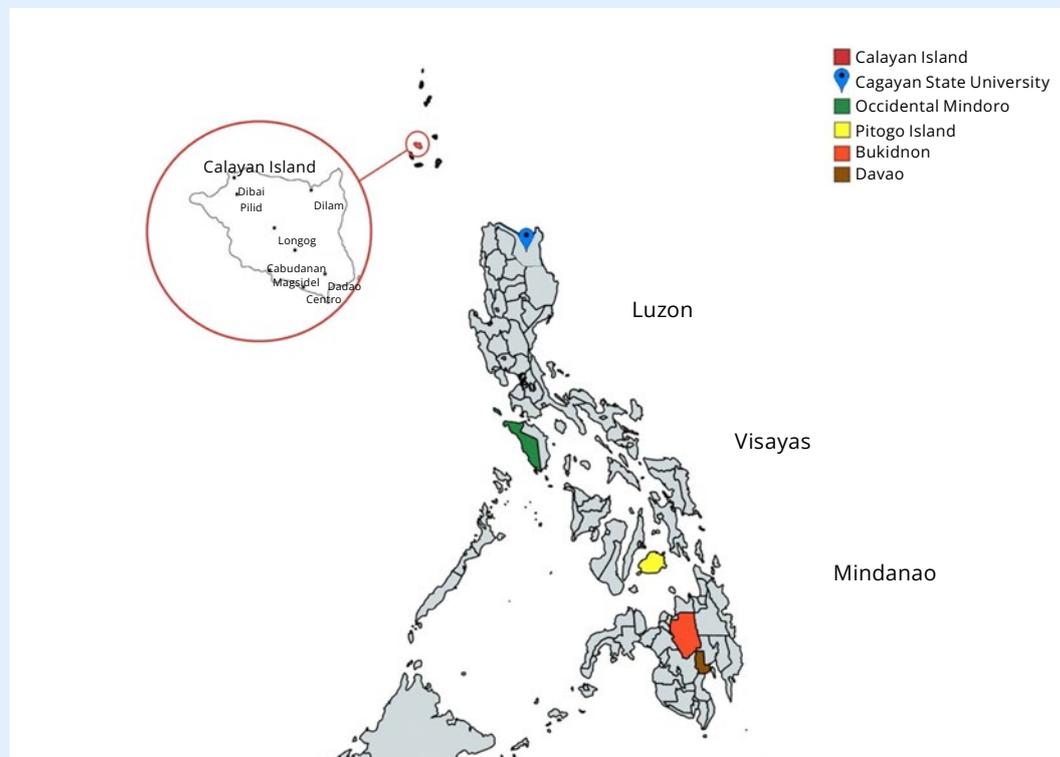
©www.mapchart.net

Different genetic markers have been used to determine the genetic variation of swamp buffaloes. These included genetic markers informative for identifying the maternal and paternal identities (Amano *et al.*, 1994; Tanaka *et al.*, 1996; Paraguas *et al.*, 2018; Kikkawa *et al.*, 1997; Villamor *et al.*, 2021a, 2021b; Cailipan and Villamor, 2021). Microsatellite markers can be informative in determining water buffaloes' genetic diversity (Zhang *et al.,* 2007).

The project

The project "Genetic Diversity of the Philippine Carabao Using mtDNA (COI) and Microsatellite Markers (FAO STRs)" was funded by the Department of Agriculture – Bureau of Agricultural Research and Biotechnology Program. It was implemented by the National Headquarters and Gene Pool of the Philippine Carabao Center (PCC). From 2015 to 2021, the project was focused on finding the distinct and pure type of Philippine carabao for conservation management.

In the past, FAO has recommended the use of microsatellite panels consisting of 30 markers for water buffalo population diversity analysis (FAO, 2011). But these genetic markers needed to be optimized in the Philippine swamp buffalo populations, using molecular techniques, before utilization. The necessary steps were:

1. Site selection and blood collection

The collection sites were coordinated and facilitated by the PCC regional manager and government units. The site collection considered highly isolated areas such as the mountainous and coastal parts of the country to help ensure no introduction of artificial insemination programme of the riverine buffaloes had been performed. In this study, 139 fresh blood samples of Philippine carabao were collected from six sub-populations from: (i) Luzon: Calayan Island (n=35), Cagayan State University (n=21) and Occidental Mindoro (n=13) (ii) Visayas: Pitogo Island (n=35) and (iii) Mindanao: Davao (n=20) and Bukidnon (n=15) (Figure 2). Five riverine buffaloes from the PCC National Headquarters and Gene Pool were included as an outgroup. The Philippine Government had in the past imported riverine buffaloes from Brazil and India to cross them with swamp buffalo populations in the country to increase the incomes of smallholder farmers.

2. Deoxyribonucleic acid (DNA) extraction

Hereditary material from genomic DNA was extracted from whole blood samples using a commercially available DNA extraction kit (Promega ReliaPrep™), with little modifications to the manufacturer's recommended procedure (Villamor *et al.*, 2021a)

3. Polymerase chain reaction (PCR) optimization

The study was based on the panel of 30 microsatellite DNA markers selected by members of the International Society of Animal Genetics (ISAG) in cooperation with FAO (FAO, 2011). The suggested 30 ISAG/FAO microsatellite markers for buffalo were optimized and applied to the Philippine carabao populations by the National Livestock Cryobank of the PCC.

The single-plex PCR and PCR thermal cycling conditions for all 30 microsatellite markers were optimized (Escuadro and Villamor, 2021). However, the genetic variation of buffaloes differs from different populations worldwide. Hence, reference genotypes of Philippine swamp buffaloes' sub-populations need to be established. This led to using single-plex PCR validation across 30 microsatellite markers.

4. Fragment analysis

Amplified PCR products were sent to an international service facility for fragment analysis. All loci with at least a 95 percent success rate of amplified PCR products was included in the analysis.

5. Data analysis

The population differentiation and probability of an individual belonging to population were performed (Pritchard, Stephens and Donnelly, 2000). The optimum K value (which corresponds to the putative number of genetically distinct populations) or clustering was visualized and determined using the STRUCTURE Harvester program (Earl and vonHoldt, 2012).

The possible number of genetic groups that constitute the six Philippine Carabao populations and riverine buffaloes as outgroups was visualized and generated by the model-based clustering method (Earl and vonHoldt, 2012). Data revealed genetic differentiation in Philippine carabao sub-populations, which are divided into purebred swamp buffaloes and hybridization within-swamp buffalo type.

Results

Figure 3 shows the results of the model-based clustering analysis. The model had the best fit when K=4, suggesting the existence of four sub-populations that contributed to the genetic make-up of the animals studied. The four sub-populations include the Calayan Island, Pitogo Island, the hybrid of swamp buffaloes from Calayan Island and Pitogo Island (Cagayan, Occidental Mindoro, Davao and Bukidnon), and the outgroup from riverine population (PCC institutional herd).

The identified swamp buffalo sub-populations in Calayan Island and Pitogo Island are distinct clusters and purebred swamp buffaloes. The Pitogo Island buffaloes seem to show substantial common ancestry with the Davao and Bukidnon sub-populations, whereas the Cagayan buffaloes show more commonality with the Calayan Island sub-population. Pitogo Island and Calayan Island are isolated islands located about 30 minutes (Talibon to Pitogo Island) and five hours (Aparri to Calayan Island) by boat from the mainland. Physical barriers caused by great bodies of water impede genetic exchanges between the neighbouring sub-populations, most likely resulting in reproductive isolation. In addition, the respective local government units institutionalized local ordinances for establishing the carabao sanctuaries in Calayan Island and Pitogo Island.

Buffaloes from Cagayan State University showed closer affinity to Calayan Island than other swamp populations. This could be explained by the proximity and accessibility of Calayan Island to Cagayan. On the other hand, swamp buffaloes from Occidental Mindoro elucidated a closer

Figure 3. K=2 to K=5 plot estimate of population structure of six swamp buffalo sub-populations, with riverine buffaloes (R) as an outgroup. An individual sample is represented by a thin vertical line which was divided into coloured segments representing the proportional contribution of inferred clusters
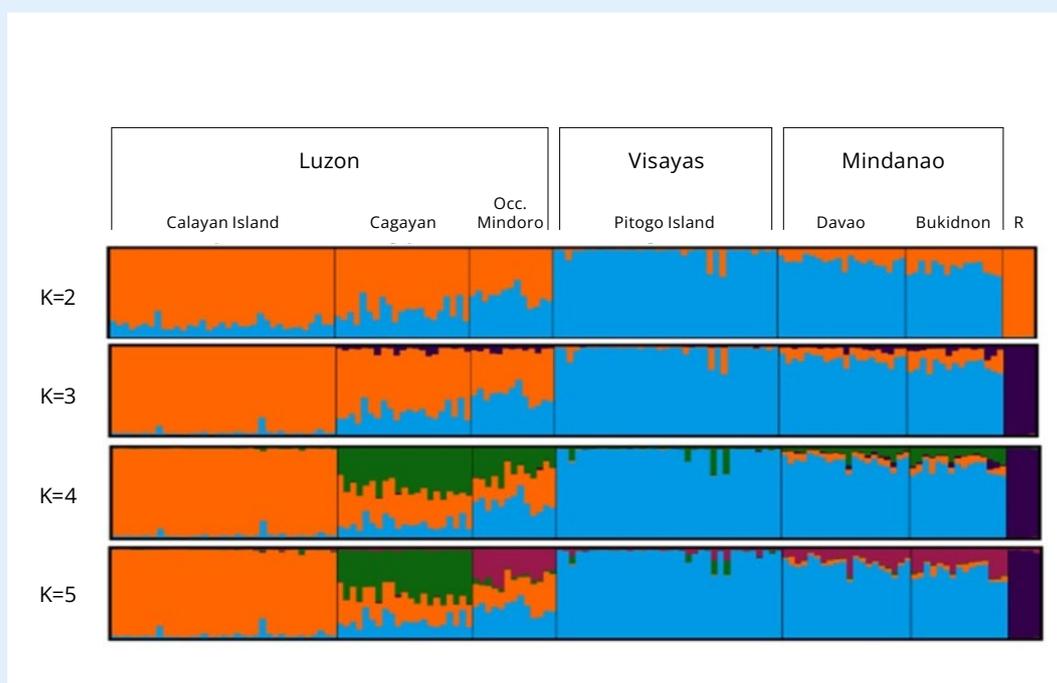
genetic relationship to Pitogo Island than Calayan Island and Cagayan State University. The high gene flow among these sub-populations could be due to the closer geographical locations and easy accessibilities through inter-island transportation from Occidental Mindoro to Pitogo Island and from Pitogo Island to Davao and Bukidnon, and vice versa.

Discussion

Challenges in use of the biotechnology

The assessment of microsatellite DNA markers showed that 27 of the 30 markers suggested by ISAG/FAO could be applied here. The three other microsatellite markers (RM099, HMHIR and ILSTS008) exhibited a single allele per locus and were non-informative for distinguishing the sub-populations.

PCR products need to be analyzed in a high-throughput DNA fragment analyzer. The initial investment and equipment maintenance are very high, so outsourcing to an international laboratory was done. However, it has the disadvantage of delayed processing, which was further worsened by the drastic effects of the COVID-19 pandemic.

Accurate fragment sizes are usually not reproducible in different laboratory settings. For example, identical fragments can appear to be different lengths when run on other machines or even different runs on the same machine, considering variations in voltage, temperature and reagents for separating the fragment lengths. Due to different laboratory settings, the microsatellite data obtained from a single provider for fragment analysis of the present study is not comparable to those from other and previously published studies. Thus, the reference databases of species' genetic information from microsatellites are unavailable in the National Center for Biotechnology Information (NCBI) Genbank for deposit and retrieval.

The farmers' appreciation of this biotechnology was a challenge due to meagre knowledge of the basic principles of molecular analysis. In addition, the technical terms commonly used in the laboratory protocols are understandably hard for farmers to visualize and familiarize.

Impacts

The microsatellite analysis provided two important pieces of genetic information about the Philippine carabao. First, water buffaloes in the Calayan Island and Pitogo Island are pure-type swamp buffaloes, and second, pure-type swamp buffalo genotypes serve as baseline information for future monitoring of the sub-species genetic diversity.

Carabao genetic diversity current status

Based on the applications of biotechnology in this study, the long-standing question of whether there is genetic diversity in the Philippine carabao has been answered with a resounding yes. Indeed, there is genetic diversity among Philippine carabao sub-populations. The two distinct clusters are Calayan Island and Pitogo Island.

Figure 4. Left: A farmer leads his swamp buffaloes along the northern coast of Calayan Island in 2015. Right: Swamp buffaloes with farm owners on Pitogo Island

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Carabao conservation going forward

The high affinity of these animals to swamp buffaloes inferred ancestry in their native tract. This was made possible through the commitment of the local farmers to conserving the breed of native carabaos in the sanctuaries in Calayan Island and Pitogo Island (Irang and Villamor, 2017; Villamor, 2018, 2019) (Figure 4).

The *ex-situ* cryo-conservation carried out through the PCC cryobanking facility serves as a biological vault of the preserved animal genetic resources for future animal management and utilization (Villamor 2015). However, the swamp buffalo genetic resources are still limited to those from the institutional herd at Cagayan State University. Hence, the valuable insights obtained from this study suggest the existence of two newly detected distinct and purebred swamp populations. Therefore, two separate conservation programmes are recommended for strategic conservation and management. The current programme includes collecting, cryopreserving and storing viable germplasm in frozen semen and embryos from swamp buffaloes for increased species' productivity, so this should be enlarged to address both distinct sub-populations.

Calayan Island conservation efforts

PCC's conservation efforts are performed in two ways: conservation by improving its economic value and conservation by preserving, cryobanking and maintaining the good genes of native carabaos. Calayan Island is a source of large swamp buffalo stocks in Northern Luzon (Figure 5). Before these animals were identified as purebred swamp buffaloes, the prevailing price was P 12 000. However, Municipal Agriculturist Vincent Tan reiterated that the declaration of the carabao sanctuary and the established DNA profiles of pure swamp-type buffaloes in 2018 helped increase the Calayan Island swamp buffalo market value from P 18 000 to P 20 000.

In April 2016, a memorandum of agreement between the Department of Agriculture and Calayan Island local government unit officials (LGU) was signed for the identified carabao sanctuary. About 165 carabaos born between 2015 and 2016 were ear tagged as part of the initial animal inventory. In 2017, 100 hectares of land were donated by the LGUs and a financial grant by the Department of Agriculture was used to construct the communal animal facility. The facility can house at least 40 Calayan Island native carabaos for breeding and conservation.

Figure 5. A swamp buffalo grazing on Calayan Island, with the Pacific Ocean in the background

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Figure 6. Left: Senior PCC researcher Lilian Villamor (first from left) and Cagayan State University centre director Franklin Rellin (seventh from left) during a visit to Mayor Alfonso Llopis (sixth from left), municipal agriculturist Lino Llopis (second from left) and other local officials on Calayan Island. Right: Mayor Joseph Llopis explaining the PCC's programme to explore the carabao's conservation to Calayan Island farmers, in 2015

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Figure 7. The animal facility to conserve swamp buffalo on Calayan Island, under construction in 2017 (left) and completed in 2022 (right)

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Pitogo Island conservation efforts

Pitogo Island (also known as President Carlos P. Garcia Island) is the island-town dedicated to the conservation of the native carabao in the Visayas area. It was selected for two reasons. First, to honour the late President Carlos P. Garcia who preferred conserving and cherishing what the nation owns. Second, for its topographical location, which features a physical barrier that protects the conservation site from the entry of exotic breeds of buffaloes.

The signed memorandum of agreement on the carabao development programme with the PCC at Ubay Stock Farm in 2010 covered the conservation, improvement and utilization of the native carabao, providing sources of genetics for natural and artificial reproduction. In 2017, the pursuit of revitalizing the previous memorandum of agreement was benchmarked on establishing DNA profiles from native carabaos on the island to assess the population's current status as a pure native carabao breed. In addition, the technical support to native carabao farmers in the island encompasses numerous services to animals such as de-worming, vitamin administration and blood screening for infectious diseases (Figure 8).

Conclusion

Developing rational conservation programmes for the Philippine carabao entails knowledge of the pattern of genetic variability within and between populations of swamp buffalo. Therefore, studying the genetic diversity is essential to determining the distribution of the Philippine swamp buffalo towards strategic Philippine carabao conservation. Biotechnology, with DNA-based microsatellite analysis, unveiled the first report on the Philippine carabao's genetic diversity, indicating two distinct and pure-type swamp buffaloes. Therefore, the reference genotypes could monitor species' genetic diversity.

Figure 8. PCC researchers and a group of swamp buffalo farmers in Bohol Province, Pitogo Island, in 2017

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2.6 Use of genetic selection and genomics to develop Streptococcosis-resistant tilapia to address disease threats in Nile tilapia aquaculture

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Background

This case study from GenoMar Genetics describes the use of recently available advanced biotechnologies to enhance selective breeding to develop tilapia resistant to Streptococcosis, a major economically important disease of Nile tilapia currently affecting farmers worldwide. Resistant fingerlings contribute to increased survival, increased feed efficiency, better growth and improved product quality, leading to higher net return for the farmers.

Nile tilapia, a warmwater species, has become one of the most important aquaculture species globally, playing a vital role in the rapid expansion of aquaculture production to meet the growing demand for aquatic food. It is farmed in a wide range of production systems, with varying degrees of intensity and requiring varying levels of investment.

Farmed Nile tilapia are susceptible to various diseases affecting aquaculture productivity. This not only threatens the huge investment of commercial farmers, but also puts the livelihoods and food security of millions of small-scale farmers and their families in the developing and underdeveloped countries at risk. For example, in the Philippines, a marked difference was seen in the net profitability of farmers by just increasing the final survival at harvest from 65 percent to 80 percent (Figure 1). This field data from the Philippines shows that the farmers can get an additional 1.29 percent net profit with every 1 percent increase in the final survival of the fish at harvest (Joshi, 2022).

Among many diseases affecting Nile tilapia aquaculture, Streptococcosis, a disease caused by the pathogenic bacteria *Streptococcus agalactiae* and *Streptococcus iniae*, is one of the most significant, having both socioeconomic and zoonotic impacts on tilapia farmers worldwide. Streptococcosis has been endemic across the world causing significant morbidity and mortality, with mortality rates over 50 percent for acute infections. Symptoms of Streptococcosis are lethargy, erratic swimming, hyper-pigmentation of the skin, bulging of eyeballs with blood, enlargement of spleen, abdominal distension and diffused haemorrhage in the operculum, around the mouth and anus, and at the base of the fins (Plumb and Hanson, 2010). The disease reduces survival, lowers product quality and negatively impacts the profitability of tilapia businesses.

Figure 1. Graph showing the net profit of farmers at different survival rates of the final harvest in the Philippines. The linear regression curve was plotted from the data obtained from different farmers without any interventions in the managemental practises followed by them

$Y = 1.29x - 58.81$
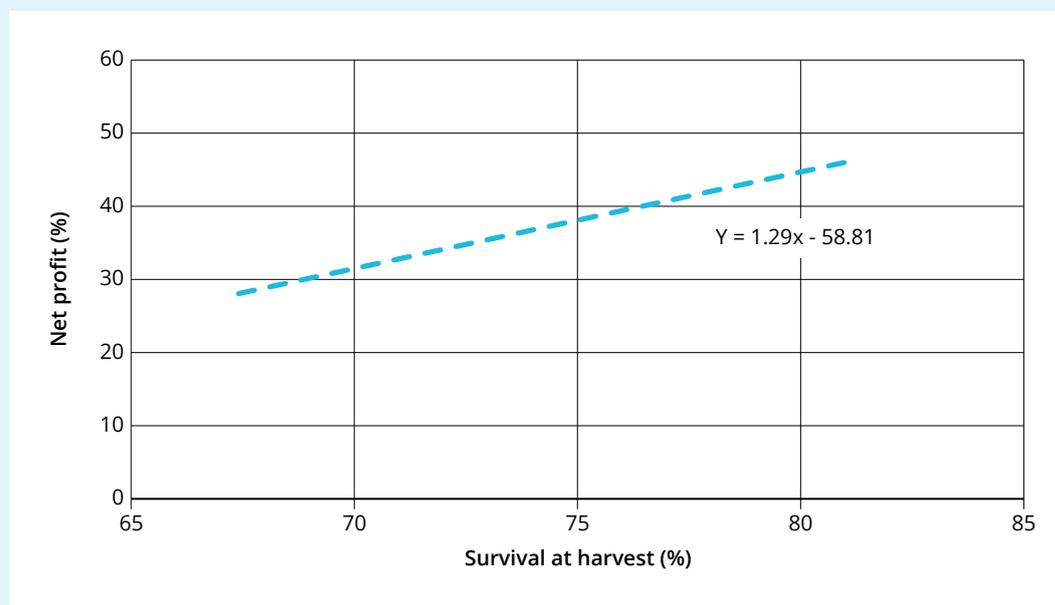
Post-infection strategy to control Streptococcosis in tilapia

In most of the world, and particularly among small- and medium-sized tilapia farmers, control measures to reduce mortality after infection are limited. Most farmers can only afford to make husbandry changes in their production systems such as reducing feeding or increasing aeration and water exchange. The use of antibiotics to control outbreaks (generally used by commercial farmers) necessitates oral treatment of the entire population of fish even though few are infected. This not only makes antibiotic therapy expensive, but also raises concerns over the development of antimicrobial resistance in both fish and humans.

Preventive vaccination strategy to control Streptococcosis

Only a few large-scale commercial farmers use vaccination to control infection and reduce mortality. Vaccination has its own challenges, especially for small- and medium-scale farmers. Vaccination by intraperitoneal injection is possible only after the fish reaches 10–15 grams, which could have an economic implication if there is any disease outbreak before vaccination. Furthermore, vaccinating large groups of fish is difficult and labour intensive, making it unattractive for small and medium-scale farmers.

Preventive genetic strategy to control Streptococcosis

Breeding animals for higher genetic tolerance or resistance to diseases is a promising innovation that has demonstrated its worth in other livestock and aquatic species. In the case of breeding for Streptococcosis-resistant tilapia, all farmers (from small-scale farmers to large-commercial farmers who have access to multiplier hatcheries) will have easy access to the improved product

without any adoption barriers as the innovation is already incorporated in the genetic makeup of the fish. Further, farmers do not need to spend additional effort or incur additional management costs because the immunity lasts for the entire lifespan of the fish. There may, of course, be a price premium for the purchase of such fingerlings compared to standard fingerlings, reflecting the additional cost of development. This is the most sustainable approach as the genetic gain for resistance to diseases is cumulative over generations of selective breeding.

Streptococcosis resistant tilapia – GenoMar Strong

Specific resistance against *Streptococcus agalactiae*, the major causative agent of Streptococcosis, was implemented as a goal in the GenoMar breeding programme in 2016 (Joshi, Skaarud and Mariussen, 2021). Since then, for each generation, fish from all families in the GenoMar breeding programme were experimentally challenged in the lab to document genetic variance in resistance to the disease. These tests showed clear and consistent variability in mortality between families, ranging from 4 percent to 75 percent, and this variability (heritability of 0.15) formed the basis for selection of families with better survival.

Early in 2021, GenoMar introduced Streptococcosis-resistant tilapia to the Asian market under the brand name GenoMar Strong, following five years of extensive R&D effort and field testing. This product was the first of its kind for Nile tilapia aquaculture, which was developed using classical pedigree-based selection. At present, the product is available to all farmers in Malaysia, Viet Nam and the Philippines through multiplication facilities located in these countries. Repeated laboratory tests and a field trial have shown an increased survival rate of approximately 30 percent for GenoMar Strong, compared to the non-selected GenoMar tilapia.

Validation of Streptococcosis resistance

The level of protection attained after three generations of selection was confirmed by an independent laboratory challenge experiment in 2019 (Joshi, Skaarud and Alvarez, 2021) and by a field trial to a commercial Malaysian farm in 2020. The results of these two experiments clearly showed increased survivability for the GenoMar Strong, compared to non-selected tilapia. The non-selected tilapia were obtained by random mating of the breeding candidates from the same population selected for growth, fillet yield and pond survival (but not directly for Streptococcosis resistance).

In the laboratory trial, cumulative mortality due to *S. agalactiae* infection in the GenoMar Strong was 28.7 percent and 32.3 percent, compared to the non-selected tilapia which was 49.7 percent and 43.0 percent when the experimental challenge conditions involved intraperitoneal injection (IP) and cohabitation with intraperitoneally injected shedder fish (cohab) respectively (Figure 2). The relative percent survival (RPS) is calculated as follows: (1−Mortality in the selected line/Mortality in the normal line) ×100. The RPS for GenoMar Strong was 42 percent for the IP challenge model and 25 percent for the cohab challenge model, compared to non-selected tilapia (Figure 2).

In the field trial, the cumulative mortality was on average 29.6 percent for GenoMar Strong and 43.4 percent for the non-selected fish after 135 days post-transfer to grow out. The dead fish showed clinical signs of Streptococcosis. This result is in line with the laboratory tests showing RPS of 32 percent (Figure 3) for GenoMar Strong, compared to non-selected tilapia. Further, Figure 3 shows that the difference in mortality between GenoMar Strong and non-selected tilapia increases gradually in later phases of production. The economic damage to the farmers is higher if the fish die

Figure 2. Cumulative mortality in a laboratory challenge test of tilapia fingerlings selected for Streptococcosis resistance (GenoMar Strong), compared to non-selected tilapia. Two different routes of infections with S. agalactiae were used to infect the fish, involving intraperitoneal injection (IP) and cohabitation with intraperitoneally injected shedder fish (cohab) challenges. Level of protection is calculated as the relative percent survival (RPS)
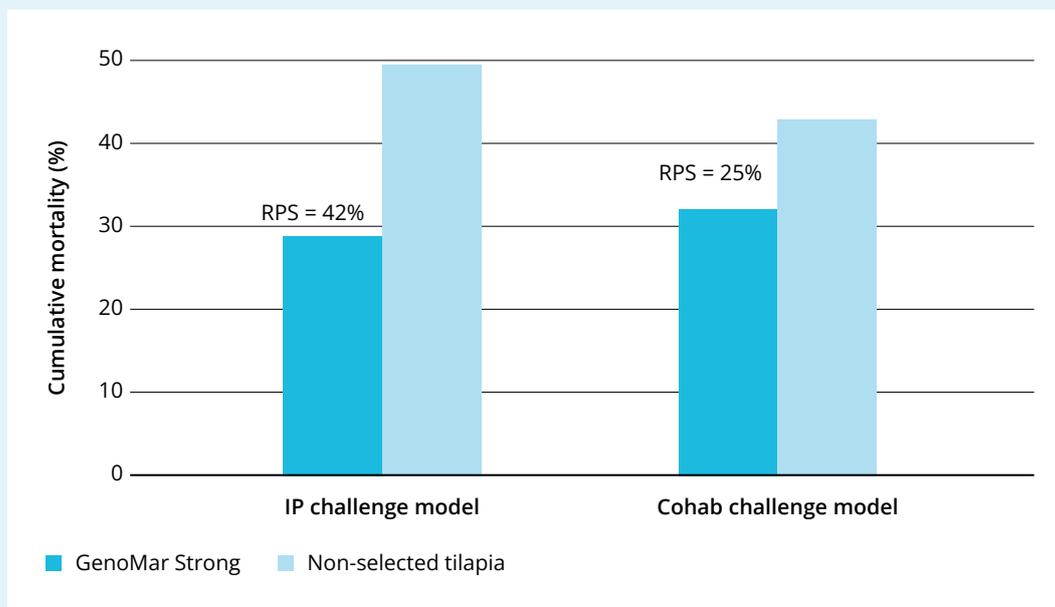
Figure 3. Daily, cumulative mortality in a field trial with tilapia selected for Streptococcosis resistance (GenoMar Strong), and non-selected tilapia, 135 days post-transfer to grow out. At a commercial farm in Malaysia, two groups of approximately 2 000 fish each were combined, individually tagged, and grown together in cages. Level of protection is calculated as the relative percent survival (RPS)
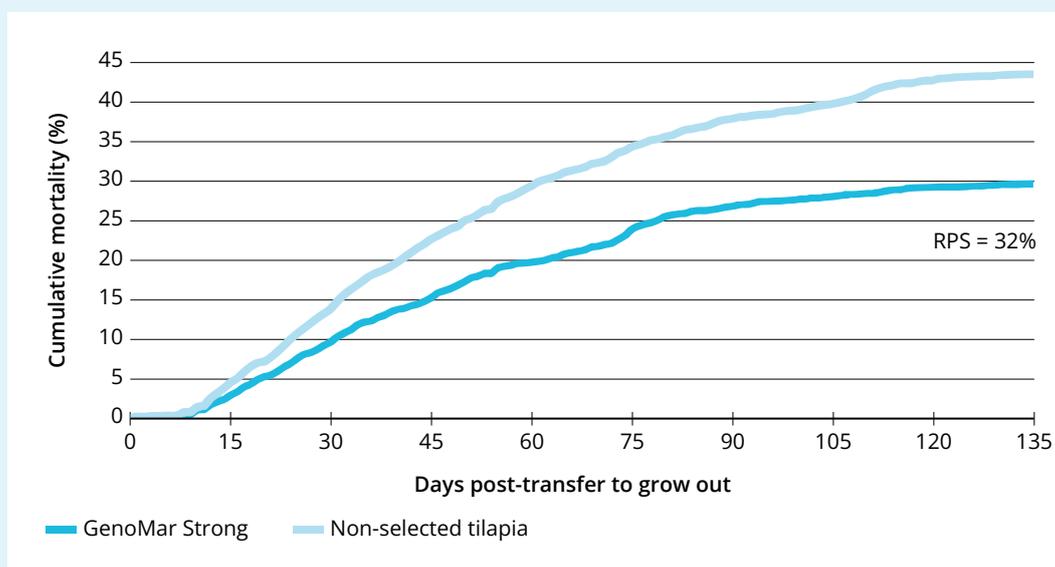
Figure 4. Break-even calculations (benefits ≥ costs) involving Streptococcosis resistant fingerlings when GenoMar Strong prices and mortalities caused by Streptococcosis increase, under pond and cage farming of tilapia in Malaysia. The green colours show the probabilities of breaking even when the benefits (protection against Streptococcosis) are higher than or equal to the costs (extra price of buying genetically selected tilapia fingerlings resistant to Streptococcosis over the standard fingerlings)
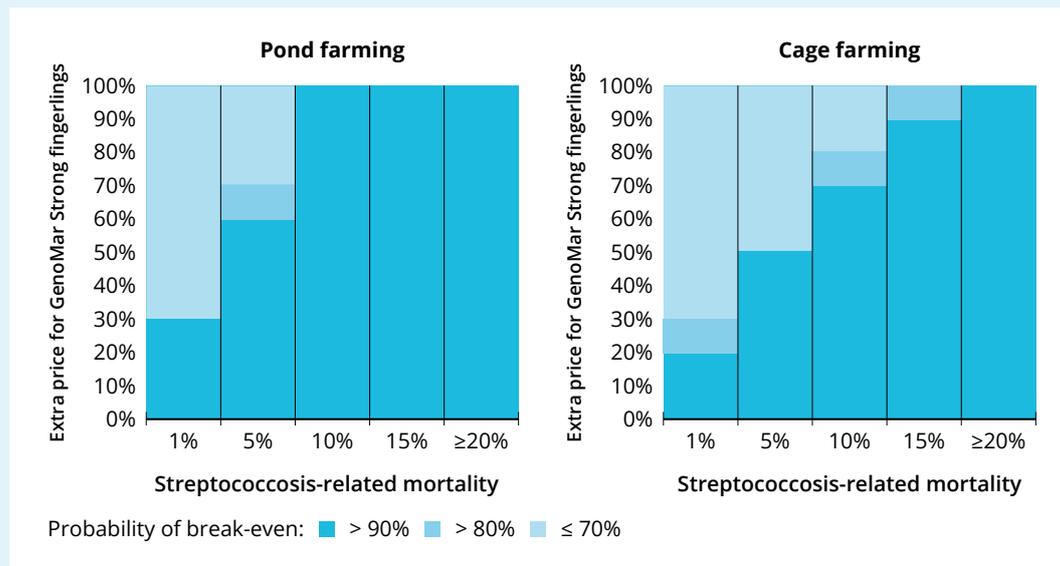
later in the grow-out phase, mainly due to the investment in feeds (in the Philippines, feed accounts for around 85 percent of the cost of production in Nile tilapia aquaculture, whereas the cost of fingerlings is around 5 percent only). This implies greater economic returns on investment using the fingerlings from GenoMar Strong.

Economic evaluation

An economic analysis was performed using data from the above field trial and data on increased survival from the published literature, to help the farmers understand the value of using such genetically selected tilapia fingerlings for production (Delphino, Joshi and Alvarez, 2022). The analysis demonstrated that farmers were expected to earn on average an extra USD 0.07 to USD 0.075 per kg of biomass harvested with GenoMar Strong in both pond and cage farming systems.

There is a price premium on the purchase of GenoMar Strong fingerlings (about 10 percent more) compared to standard fingerlings, reflecting the additional cost of development. The analysis also showed a significant higher return of investments in Streptococcosis outbreaks even if the cost of GenoMar Strong fingerlings was double that for standard fingerlings. Even with a low frequency of Streptococcosis-related mortality (1–5 percent), the net profit was in favour of GenoMar Strong fingerlings if the extra amount paid for GenoMar Strong fingerlings over the standard fingerlings was no more than 30 percent (Figure 4).

Figure 5. Data showing the proportion of the sales of Streptococcosis-resistant fingerlings (GenoMar Strong) in comparison to other products from one of the hatcheries in Asia. From mid-2022, Genomar Strong fingerlings bred from genomic selection were also sold
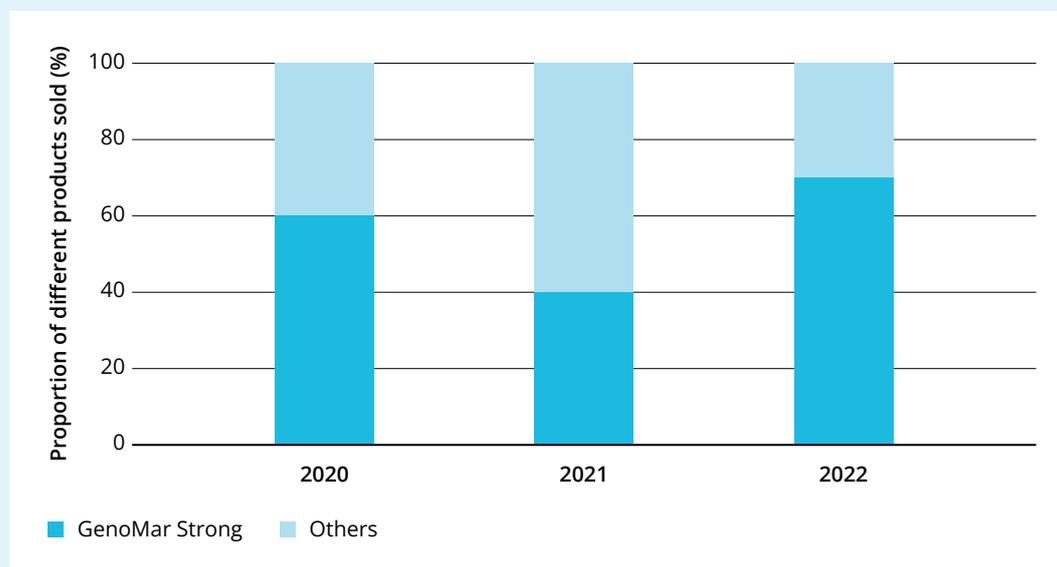
Use of genomic selection for breeding for more resilience

GenoMar developed the first commercial high-density tilapia genotyping tool, the Affymetrix SNP array (Joshi *et al.*, 2018). Information from more than 2 million places in the tilapia genome, where fish differ from each other at a single nucleotide (single nucleotide polymorphism or SNP), was used to construct the first SNP array containing 58 500 SNPs. GenoMar started using the advanced breeding technique called genomic selection using this SNP array from 2019 to provide the industry with fast-growing, robust, disease-tolerant and more productive premium Nile tilapia fingerlings.

The GenoMar Strong fingerlings developed using genomic selection were made available to the farmers in the Philippines, Malaysia and Viet Nam from mid-2022. Figure 5 shows the proportion of sales of the GenoMar Strong fingerlings from one of the hatcheries in Asia. The genomically selected fingerlings represented a significant proportion of the total number of fingerlings sold in 2022. These genomically selected fingerlings have been equally popular among tilapia farmers with all types of production systems: from small-scale production to large-scale intensive commercial production. The performance of these genomically selected fingerlings is currently being monitored in the field, with the preliminary data suggesting an average of 30 percent net profit per production cycle in the Philippines and Viet Nam.

What is genomic selection?

In genomic selection, thousands of naturally occurring mutations (called markers) present in the DNA of individual fish and complex mathematical models are both used to find out the effects of the markers that make one fish "more resistant" than another. When these effects are

Figure 6. Schematic overview of the breeding work in the GenoMar tilapia populations to select the best breeding candidates using genomic selection
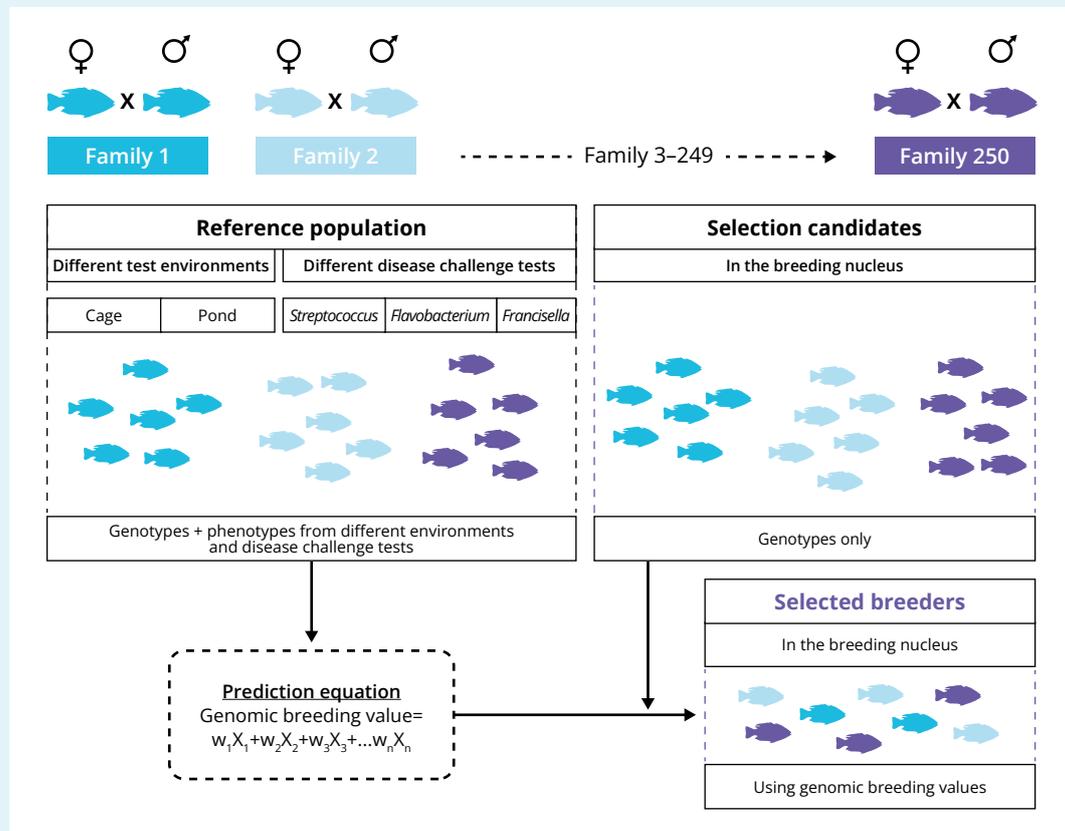
known, then the "genomic breeding values" (metrics to rank the fish) can be computed using only the DNA information from the breeding candidates (Figure 6).

The measurement of traits like resistance to Streptococcosis involves either the sacrifice of the fish or exposure to standardized pathogen challenge tests, and so cannot be done on selection candidates. As a result, challenged fish cannot be used as breeding candidates for the following generation and thus challenge tests are only performed on full siblings of future broodstock. Thus, offspring from every family in the breeding nucleus are sent to the lab and tested for resistance to *S. agalactiae,* where physical observed traits (phenotypes) and DNA-information (genotypes) are gathered for each fish. However, only DNA information is collected from the breeders in the breeding nucleus. Complex mathematical models using all these data are then used to rank the fish to pick the preferred candidates in the breeding nucleus for the next generation (Figure 6).

Advantages of genomic selection

Family-based selection limits the ability to rank the fish within the families and allows for a simple ranking of the families from best to worst. Genomic selection, however, provides a further opportunity to select the best fish within the families (precision breeding), thereby increasing the accuracy to select the best fish for breeders (Joshi, Skaarud and Mariussen, 2022) (Figure 7). We have shown that the prediction accuracy can be increased by 71 percent for

Figure 7. Prediction of breeding values of 15 individual fish in a full sib family (breeding candidates) based on pathogen challenge test results. Family-based selection only provides one breeding value for the entire family (as shown by the horizontal line). Genomic selection, instead, assigns different breeding values (genomic estimated breeding values) to each fish based on their genetic merit (as illustrated by vertical bar charts), allowing us to identify the best candidates for precision breeding
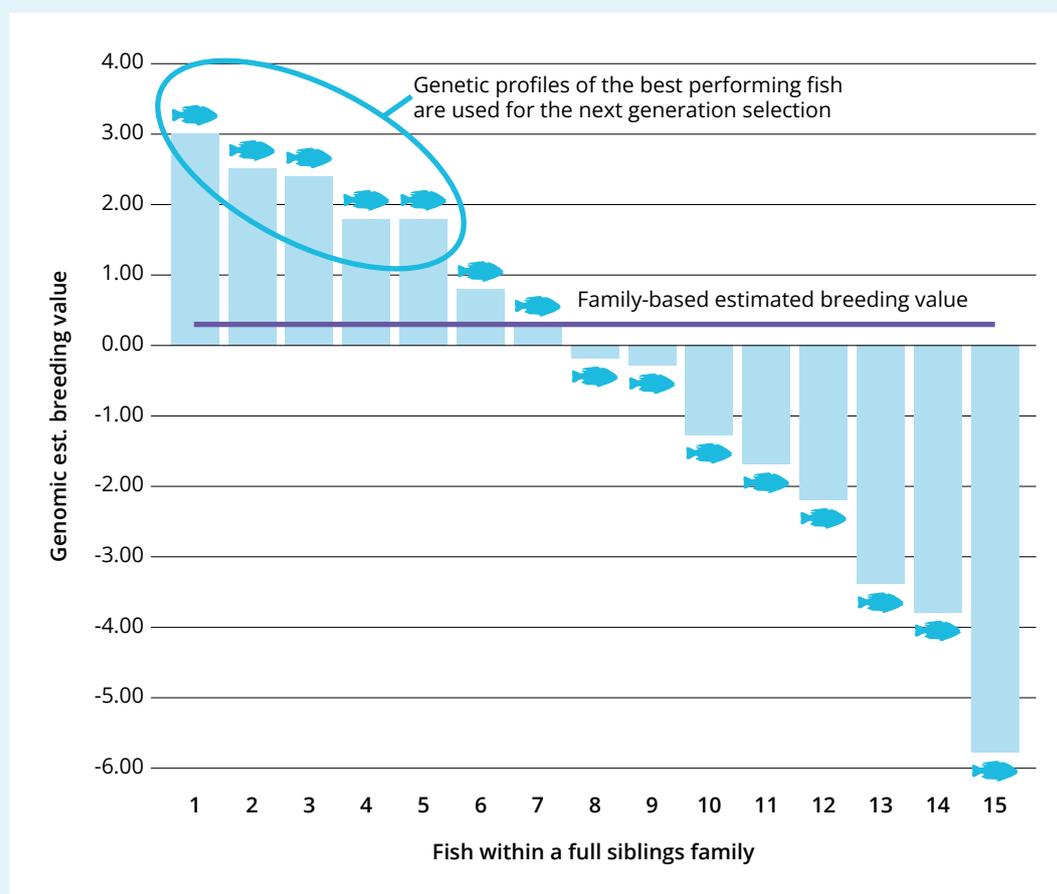
resistance to Streptococcosis using genomic selection instead of family-based selection (Joshi *et al.*, 2021). This has allowed us to have more genetic gain, and thus the fish selected using genomic selection are expected to have better survival.

Using QTLs for selection vs using genomic selection

Furthermore, DNA information from groups of resistant and susceptible fish can be analyzed using genomics so that chromosome regions associated with resistance to Streptococcosis, called quantitative trait loci (QTLs) can be identified. Genomics therefore provides the opportunity to reveal a fingerprint for higher resistance to Streptococcosis, which can be used to select the parents for the next generation of fish (called marker-based selection). This removes the need to send fish for challenge tests, thereby reducing costs of the breeding programmes.

However, the prediction accuracy of marker-based selection depends on the characteristics of the trait. If the trait is controlled by one or only few genes, then the prediction accuracy is very high. However, for traits like resistance to Streptococcosis, where the variation is explained by many genes, we have shown that genomic selection using advanced mathematical models provides better prediction accuracy (Joshi, Skaarud and Mariussen, 2022). Thus, GenoMar has been using genomic selection instead of marker-based selection to provide fingerlings with better survival to the farmers.

From breeders to the commercial fingerlings

GenoMar Genetics is an international aquaculture breeding and distribution company providing genetically improved tilapia to farmers globally. In the tilapia value chain, selective breeding happens in the breeding nucleus whereas the commercial fingerlings are produced in the hatcheries after undergoing a series of systematic matings in multiplier units. The top ranked breeders from the GenoMar breeding programme (also called great grandparents) are selected and systematically mated for three generations (great grandparents to grandparents to parents to commercial fingerlings) in multiplication units to produce the commercial GenoMar Strong fingerlings (Joshi, Skaarud and Mariussen, 2021).

Subsidiary companies of GenoMar dealing with different components of the tilapia value chain are strategically present in various parts of the world for easy distribution of the fingerlings to the farmers. GenoMar has its head office and central R&D office in Norway from where all the breeding, multiplication and marketing activities are monitored. Selective breeding in multiple breeding programmes happens in the state-of-the-art breeding nuclei located in the Philippines and Brazil. The first phase of the multiplication (great grandparents to the parents) is done inside these breeding nuclei. For the second phase of multiplication parents are sent to the GenoMar hatcheries in different countries. At present, GenoMar has hatcheries in Asia (in the Philippines, Malaysia and Viet Nam) and in Latin America (in Brazil and Colombia). Parents are mated in these hatcheries and fingerlings are produced and distributed to the farmers.

Besides all the technologies being used for genetic selection, which has been described in this document, the way we select these great grandparents (breeders) directly affects the level of protection against Streptococcosis in the commercial fingerlings. Selecting the very few top ranked breeders (great grandparents) for creation of the product allows us to provide the farmers with the commercial fingerlings having the best possible protection against Streptococcosis (Figure 8). Selecting the top 0.5 percent breeders, as shown in Figure 8, provides us with the opportunity to produce the GenoMar Strong fingerlings with 81 percent higher genetic potential (mean estimated breeding value) compared to the method by which most of the suppliers in the industry create the commercial fingerlings (all breeders method using the most resistant families, as shown in Figure 8). This also provides a degree of protection of the company's intellectual property, given that inbreeding will rapidly accumulate if crossing and/or further breeding is carried out using commercial products released for grow out.

Figure 8. Comparison of the mean scaled estimated breeding values of the tilapia breeders (great grandparents from the GenoMar breeding programme) used to create the Streptococcosis-resistant fingerlings using three different methods. GenoMar uses the top 0.5% breeders from the generation who are systematically mated to produce the commercial fingerlings, GenoMar Strong
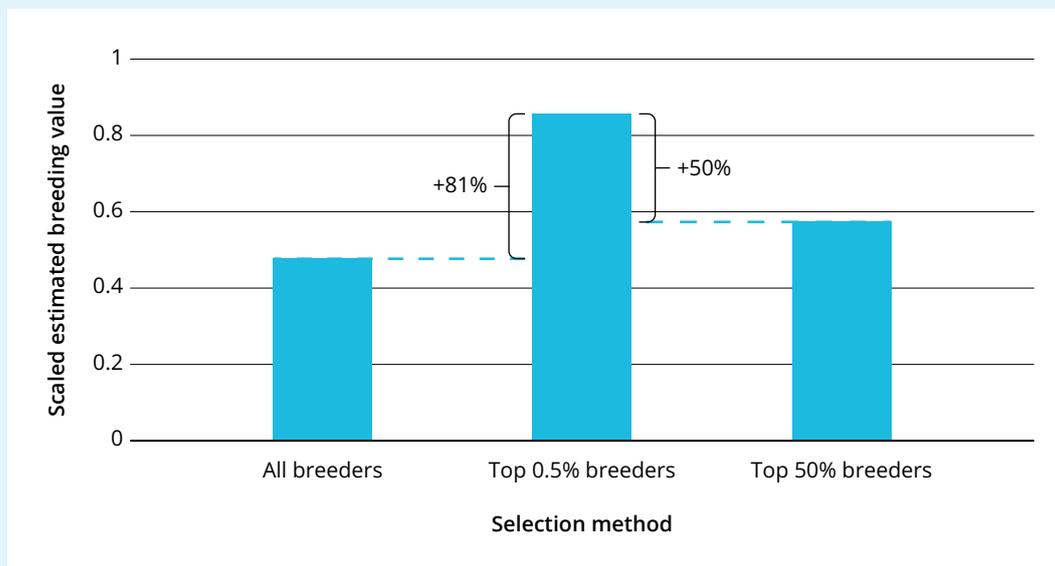
Conclusion

With the use of advanced technologies such as genomics and the expansion of breeding goals and phenotyping to novel health and resilience-related traits, Nile tilapia breeding programmes are becoming increasingly complex. This paper has described the case study for such a product.

As has occurred in other major food species such as chicken and pigs, specialization and separation of players across the value chain (primary breeders, producers, processors, etc.) is an enabling factor in this development, as advanced breeding programmes require specialized resources and the need to be well financed, long term and scalable.

Using genetically selected fingerlings like GenoMar Strong reduces the risks of losses due to Streptococcosis, offering the possibility of better harvests with significantly increased income to the farmers. In addition to these direct benefits there are also social and environmental benefits of using such products, including improved overall fish resilience, a reduced use of antibiotics, lower generation of mortality waste and higher resource efficiency. All these contributions will support the development of a more sustainable Nile tilapia value chain.

Photo 1. Streptococcosis-resistant GenoMar Strong fingerlings at the hatchery in the Philippines

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Photo 2. Industrial scale comparison of GenoMar Strong and non-selected tilapia in cages in a commercial Malaysian farm

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Photo 3. GenoMar Strong juveniles at the hatchery in Viet Nam

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Chapter 3: Case studies in crops and forestry

3.1 The use of the sterile insect technique as part of an integrated pest management approach to control fruit flies in Ecuador

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Abstract

In Ecuador, the production of fruits, including non-traditional fruits such as dragon fruit (pitahaya), golden berry (uvilla) and tree tomato, is on the rise. Nevertheless, the presence of a complex of fruit flies, where the Mediterranean fruit fly is the predominant species, causes damage and restricts the expansion of commercialization of these fruits to other high value international markets. Countries that are free from fruit fly pests apply quarantine regulations to fruits grown in areas where some of these fruit fly species are present. With assistance from FAO and IAEA, Ecuador has been able to implement a fruit fly pest control scheme based on integrated pest management (IPM). This has allowed the implementation of IPM over 10 700 hectares of commercial fruit production, significantly reducing fruit fly damage and increasing yields. In 2018, the sterile insect technique (SIT) was introduced to Ecuador and integrated to the IPM approach specifically against the Mediterranean fruit fly. For the implementation of SIT, 3 million sterile Mediterranean fruit flies were shipped weekly from Guatemala, packed and released in the field over the targeted production areas within the total area under IPM practices. This approach has allowed the establishment of fruit fly low prevalence areas to reduce the risk of fruit infestation. As a result of this integrated effort and pest risk mitigation schemes, fruit production and quality has increased and, for the first time ever, the export market of the United States of America has opened to these non-traditional fruits.

Introduction

The horticultural sector in Ecuador

In Ecuador, fruit cultivation (excluding bananas) covers about 163 000 hectares and involves about 120 000 farmers (William *et al.*, 2016), which is about 5 percent of arable land under cultivation in the country annually. The fruit and vegetable sector is growing and is responsible for 16 percent of the country's agricultural GDP, without accounting for the production of potatoes and bananas.

The country is the world's largest producer of banana, and generates substantial revenues from mango, coffee and pineapple. Additionally, non-traditional fruits such as dragon fruit (pitahaya), golden berry (uvilla) and tree tomato are also grown in the country, of which more than USD 314 million have been exported since 2018, including to markets that require fruit fly free products. There is significant potential for the increased cultivation of these non-traditional fruits due to both the growing international demand and the favourable agricultural conditions in the country.

Fruit flies

Fruit flies constitute the most serious constraint to the production of fresh fruits in Ecuador, the subsequent export of the produce to other countries and, hence, the further development of the horticultural industry in the country. The fruit flies of major economic importance in Ecuador belong to the genus *Anastrepha* (in particular *A. fraterculus*) which are endemic to the American continent, and the non-native Mediterranean fruit fly or medfly (*Ceratitis capitata*, Wied.) (Tigrero 1998). The medfly was introduced into Brazil in 1901 and has since spread to most of the countries in South and Central America. Fruit fly pests have been traditionally controlled by farmers using conventional insecticides. However, in most cases, fruit damage remains significant (Vilatuña *et al.*, 2016). In addition, the excessive use of insecticides has had a negative impact on the populations of natural enemies, causing outbreaks of secondary pests and resulting in further fruit damage and increased control costs. Furthermore, the presence in an area of fruit flies of quarantine concern to the importing country, such as the medfly, even when they are present at low population levels and even when the pest is not considered a primary pest of the fruit crops cultivated in the area (as is the case for Ecuador), will limit the possibilities for exports of the fruit commodities. Phytosanitary schemes that can be applied to reduce the pest risk to an acceptable level for the importing country include the establishment and maintenance of low pest prevalence and pest-free areas (FAO, 2016, 2019).

The sterile insect technique

The mass rearing and sterilization of male insects, which are subsequently released over areas where the same insect species is a pest, is known as the sterile insect technique (SIT). When the released sterile males mate with fertile females of the pest population, the reproduction of the pest is inhibited as the females fail to produce offspring. SIT is therefore an insect birth control technique. When sterile males are continuously released using, for example, a ratio of ten sterile males to one wild female, the pest population is gradually reduced each generation until the pest is suppressed and eventually can be eradicated from the area (Dyck, Hendrichs and Robinson, 2021).

In the case of fruit flies, effective application of SIT requires the release of sterile males only. For this purpose, breeding colonies at the mass rearing facilities use genetic sexing strains (GSS), developed through classical genetics, allowing the elimination of the females during the mass rearing process. In the case of the medfly, the GSS strain has the characteristic that females are sensitive to heat, thus they are eliminated very early in the rearing process by increasing the temperature during the egg incubation stage. The remaining male eggs are seeded in an artificial organic diet that is rich in proteins and carbohydrates.

After egg hatching, larvae feed on the diet and, when fully developed, are separated from the rearing substrate for pupation. Fully mature pupae are tagged with a natural fluorescent dye before they are exposed to low doses of radiation to sterilize the males. Today, the most common radiation source used for insect sterilization is gamma ray, with X-ray technology being developed as an alternative. Sterile pupae are transported in containers with controlled temperature and humidity to facilities

Figure 1. Sterile fly release box being loaded onto an aircraft

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where they are prepared for emergence of the male adult flies. Adult sterile male flies are fed for three to five days until they reach sexual maturity. Before adult flies are released in the field, they are immobilized using cold temperatures and placed in special boxes. The boxes are loaded in small aircrafts for aerial release (Figure 1). The aircraft flies over target areas where the pest is present and releases the sterile males following predetermined flight lanes. In successive flights, the sterile male flies are released over the target areas until suppression or eradication of the pest is achieved.

SIT is an effective and environmentally friendly method of pest control. It is applied on an area-wide basis and its effectiveness is maximized when applied as part of an integrated pest-management approach (Hendrichs, Pereira and Vreysen, 2021). As the technology requires an area-wide application covering the total pest population in an area, as opposed to controlling the pest on an orchard-by-orchard basis, the active involvement and coordination of all relevant stakeholders, including the general public, becomes an important requirement and a major challenge.

The control of fruit flies in Ecuador using SIT

The protocols for the use of SIT to control medfly were developed in the 1970s at the Insect Pest Control Laboratory of the Joint Food and Agriculture Organization of the United Nations (FAO)/ International Atomic Energy Agency (IAEA) Centre of Nuclear Techniques in Food and Agriculture, Seibersdorf, Austria. Through a technical cooperation project that initiated in 2016, funded by the IAEA and implemented by the Phytosanitary and Zoosanitary Regulation and Control Agency (Agrocalidad), institutional and human capacities were strengthened in Ecuador for the use of SIT to

Figure 2. Capacity building by supplying fruit fly identification equipment and training in support of project activities. Here, people are being trained in fruit fly identification

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control medfly. The activities that were implemented included a technical and economic feasibility study, expert on-site missions, the training of technical and professional staff, supply of specific materials and equipment (Figure 2) and assistance for the preparation of a technical dossier for the recognition of the fruit fly-free and low-prevalence areas.

In Ecuador, the National Fruit Fly Project (PNMMF), which was aimed at the control of medfly by establishing and maintaining fruit fly free and low prevalence areas in the country, started in 2014. The project was funded by the Ecuadorian State with investment funds for 6 years. The use of SIT as part of an integrated pest management scheme to control medfly infestation was conceived as a cost-effective and environmentally friendly pest management practice – due to the expected reduction in fruit damage and in the use of insecticides. It was also considered a sustainable means for Ecuadorian fresh produce to be traded in international markets and, thereby, enhance income generation for the farmers and other stakeholders along the producers value chains.

In November 2018, sterile male medflies were shipped to Ecuador from a large-scale mass rearing and sterilization facility located in Guatemala. Three million sterile male medflies were shipped every week to the airport in Quito, Ecuador, from where they were transported to a packing and release facility in Tumbaco, a parish located in the Pichincha Province. Sterile males were retained in the facility until sexually mature, at which point they were transported in refrigerated vehicles to the areas where medflies were present in commercial fruit plantations and released into the environment.

Figure 3. Mediterranean fruit fly values expressed in fertile flies per trap per day (FTD), in the fruit production area of Nanegal, Pichincha during 2018 and 2019
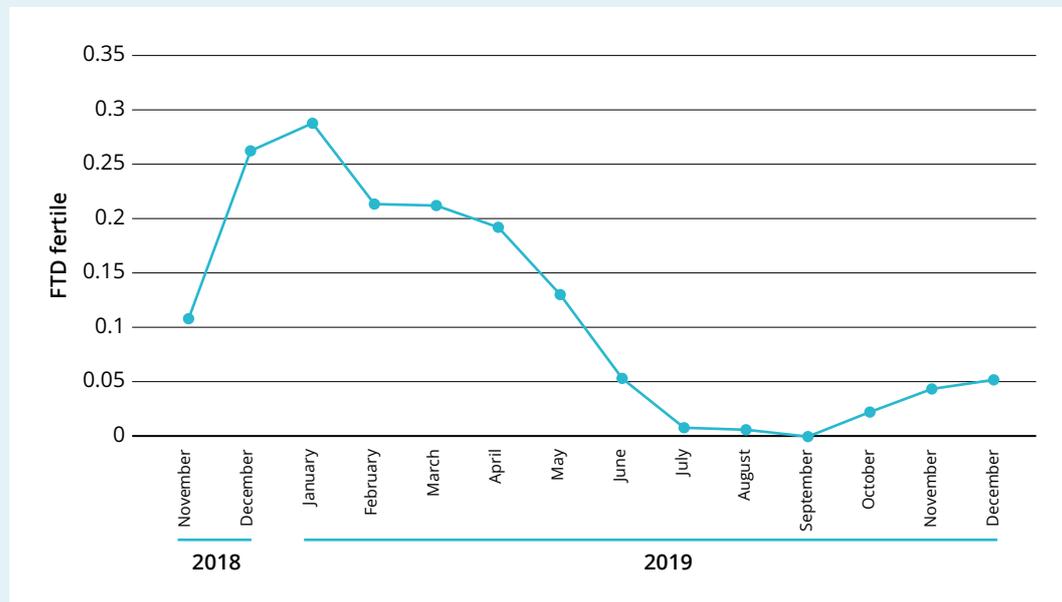
There were weekly releases in the provinces of Pichincha, Imbabura and Tungurahua, where most of the growers were small and medium holders. The pest populations were monitored by deploying a trapping network in and around the commercial fruit plantations. Results of this action showed that medfly populations were kept at low prevalence levels without the use of insecticides sprayed in open field (Figure 3). A very low amount of insecticide was used in bait stations, small containers placed on the fruit trees with bait to attract flies and an insecticide to kill them. However, there was no insecticide sprayed in the environment. Furthermore, no infestations were found in fruit by the inspectors of the importing countries, which certified that fruits were pest free and safe for trade.

Impact of integrating SIT to manage fruit fly populations on the commercial fruit industry in Ecuador

Through this project, Ecuador was able to realize the set objective of increasing and diversifying fruit production for the national and export markets by integrating SIT to a fruit fly management scheme allowing for the maintenance of low fruit fly population levels in a sustainable way. This was because, as a result of maintaining medfly populations at low prevalence levels in fruit plantations in Ecuador, the exportation of dragon fruit to the United States of America was approved for the first time in 2017. Over 2 000 tonnes was exported there in 2018 and over 3 000 tonnes in 2019 (Figures 4 and 5). This was followed soon afterwards in those respective years with approvals for the importation of tree tomato and then golden berry into the country (Figure 6). The value of these exports from Ecuador was estimated at USD 22 million per annum. The enhanced production and exportation also resulted in the creation of jobs along the value chains. Moreover, negotiations with the national plant protection organizations of other countries to permit the exportation of these and other products from these areas was initiated in Argentina, Brazil, Colombia, Peru, Japan and China (Rendón and Enkerlin, 2020).

Figure 4. Dragon fruit (*Hylocereus sp*.) in a box ready for export

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Figure 5. Exports of dragon fruit to the United States of America

Dragon fruit (Tonnes)
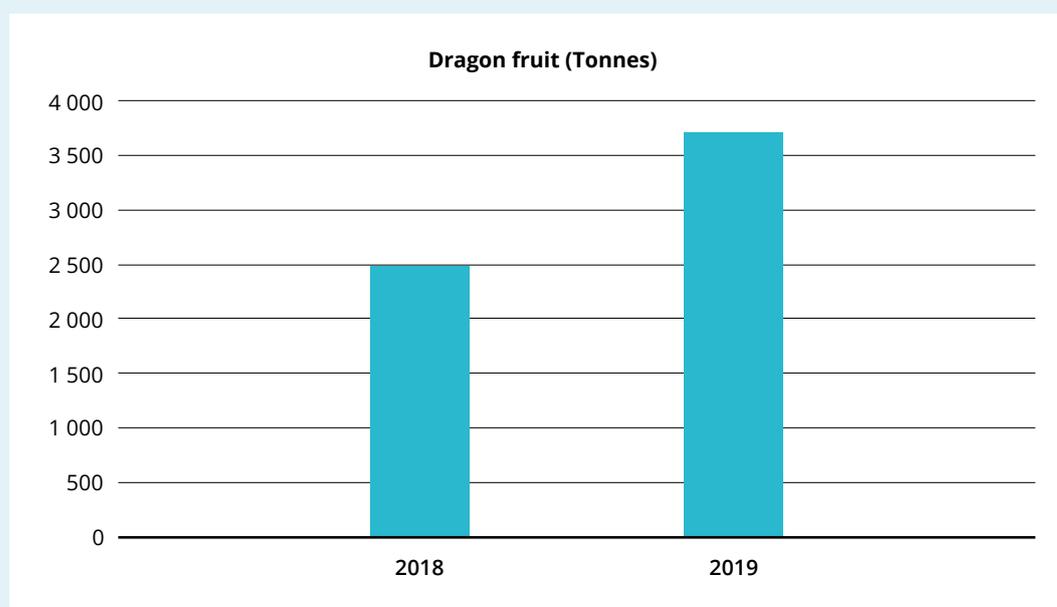
Figure 6. Tree tomato (*Solanum betaceum*) being harvested in fruit fly low prevalence areas in the highlands of Ecuador

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Elements of success

The successful adoption of SIT for the control of medfly in Ecuador was made possible by the partnership between Agrocalidad, IAEA, FAO and the fruit growers, which was underpinned by a shared objective. The partnership allowed the concerted investment of efforts and the pooling of resources. In this regard, a long-term commitment, coordination and active participation of these different stakeholders, including the organized growers, was critically important. The capacity building conducted through the FAO and IAEA technical cooperation project and the availability of an external source of sterile fruit flies was crucial in the feasibility of adopting this technology in Ecuador.

Challenges of applying SIT and lessons learned

For the National Fruit Fly Programme in Ecuador, the implementation of the SIT with the support of the IAEA and FAO constituted an important milestone. However, programme operations over a period of time presented technical challenges necessary for the optimization of the technology. These included the need for continuous sterile fly releases, expanding the release areas to benefit from economies of scale, increasing the capacity to handle a greater number of sterile pupae, and adoption of the chilled adult release method. This is tied with the search for programme sustainability in the coming years including budget allocation and fostering new strategic partners.

Lessons learned from the application of the SIT include IAEA and FAO international cooperation as a fundamental axis to venture into a new technology that became a successful case through strengthening the phytosanitary surveillance mechanisms and pest management practices. Another essential lesson was the need to establish strategic alliances with fruit growers and exporters, who are the beneficiaries, as well as with other relevant stakeholders that can contribute to the sustainability of the programme. The dissemination of information and adequate communication of the activities conducted by the programme to the beneficiaries and the general public, as well as the benefits obtained through an improved pest management scheme, also proved to be an essential lesson for the successful application of the SIT.

Future perspectives

Ecuador has recently established a National Project, following up on the PNMMF, to continue developing fruit fly low prevalence and free areas. With this, it is expected that the areas of the country in which integrated management strategies, which include an SIT component, are used will expand, not only in selected areas in the highlands but also to the fruit production areas in the coast where mango is commercially grown. It is also expected that, with the use of SIT for the control of medfly proof of concept having been demonstrated in Ecuador, the technology could be further used to control the other fruit fly species of economic importance, such as the South American fruit fly (*A. fraterculus*), in the country and possibly in many other countries in the region.

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3.2 Impact of induced genetic variation and genomics technologies on rice and soybean productivity in Viet Nam

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Abstract

The production of rice, a major food security crop in Viet Nam, was constrained by low productivity – owing in large part to the cultivation of unimproved varieties of the crop. The domestic production of soybean was likewise insufficient in meeting consumer demand – necessitating the unsustainable expenditure of foreign exchange on the importation of the crop. The exposure of the seeds or other plant propagules to physical irradiation or chemical mutagens induces novel genetic variation, which could be used in the development of superior crop varieties. Precise selection processes, sometimes with the application of molecular markers, and a streamlined breeding scheme can enhance the efficiency of the development of improved varieties following induced mutagenesis. The practice of mutation breeding in Viet Nam since the 1980s resulted in the development of 80 commercialized mutant varieties, including 54 of rice and 16 of soybean. The Agricultural Genetics Institute (AGI) of Viet Nam is a leader in mutation breeding in the country and has commercialized 19 mutant varieties of rice and 13 of soybean. The earliest released rice mutant variety, DT10, has recorded a cumulative area of 2.4 million ha since its release, contributing to an increased income of USD 536 million and benefiting 3 million farmers. The most recently released rice mutant varieties included DT80, CNC11 and VDT99. The DT80 variety, for which the presence of the *Saltol* QTL was confirmed through marker-assisted selection, can withstand saline soils and has a cumulative area of 15 000 ha since its release in 2019. It has an average yield of 6 to 7 tonnes per hectare, leading to increased earnings of USD 330 per hectare compared to its parent. Mutation breeding is also routinely applied to the genetic improvement of soybean with over 50 percent of the area under soybean cultivation in the country planted to mutant varieties. High-yielding, short duration varieties are sought, with the mutant variety DT 2008 cultivated by about 1.5 million farmers. With increasing demand for black soybean for food processing, with DT 215 the only black-seeded variety in Viet Nam, research is in progress to determine the molecular basis for the trait, with initial focus on known genetic loci associated with the trait to facilitate molecular breeding.

Introduction

The economy of Viet Nam is largely based on agriculture. The sector employed 18 million Vietnamese in 2020 and is the largest among the economic sectors of the country. The estimated agricultural area of the country is 12.4 million hectares (ha), about 40 percent of the total land area of 31.3 million ha. The rural agricultural population constitutes over 60 percent of the country's 98.5 million total population. Smallholder agriculture predominates, with average land holdings below one hectare, which are among the smallest in the world.

Rice

Rice is the main food in Viet Nam, with an average consumption of 145 kg per person per year, constituting 90 percent of all food consumed in the country. About 3.8 million hectares (ha) of cropland are restricted to rice cultivation to ensure food security (Hoang *et al.*, 2021). Although grown in all parts of the country, the two major rice producing areas are the Mekong River Delta in the south and the Red River Delta in the north, where the crop is grown annually on 2 million and 1 million ha respectively. While the land area under rice cultivation annually is 3.8 million ha, the actual harvested area is 7.9 million ha, resulting from the cultivation of two to three crops per year.

Chronic hunger and widespread poverty prevailed prior to the 1980s. The situation was gradually reversed when Viet Nam achieved rice self-sufficiency in 1989, and later became the second largest exporter of rice by 1997 (López Jerez, 2020; Figure 1). The economic and liberalization reforms of 1981 and 1988 with policy targets of food security and expansion of rice production drove this change. Average annual yield and production of rice that were, respectively, at a low 2.4 tonnes per ha and stagnant at about 11 million tonnes during 1976–1980 increased by 3.2 percent and 3.1 percent respectively during 1981–87 (Young *et al.*, 2002). Production increased again by an average of 5.2 percent annually during 1988–1995, 2.4 percent of which resulted from area expansion and 2.8 percent from yield increases. A further 20 percent rise in production from 1995 to 1999 resulted from 13 percent area and 7 percent yield increases.

These increases were made possible by the widespread adoption and cultivation of improved higher yielding and shorter duration rice varieties. Higher yields contributed to direct production increases, and shorter duration facilitated two or three cropping seasons per year, rather than just one.

Soybean

Soybean is a traditional food crop in Viet Nam, and its increasing current demand includes utilization for livestock feed. Domestic soybean production meets only 8 to 10 percent of demand for the crop, making it the country's second largest imported commodity after maize. The domestic production of the crop is constrained by price competition with imported soybean, as well as by the small and fragmented land holdings. The crop's average yields have traditionally been low – at 0.68 tonnes per hectare in 1980.

The genetic improvement of the country's crop through induced mutations commenced in the late 1980s. The crop's yield increased to an average of 1.5 tonnes per ha in 2016, with a majority of the area under soybean cultivation planted to mutant varieties, characterized by high yields, wide adaptability and the possibility of raising three crops a year (Le and Pham, 2021). The varieties with high and stable yields assume critical significance to sustaining food security, farmer livelihoods and the national economy – more so as opportunities for expansion of land under cultivation are limited and climate change poses increasing constraints to crop production systems.

Climate change

Climate change is a serious threat to Viet Nam, and drought, salinity and an increase in incidences of diseases and pests resulting from global warming remain challenges to both rice and soybean production, and hence to the livelihoods of smallholder farmers. A rise in sea level of 1 m can partially inundate 11 percent of the population and 7 percent of agricultural land (Bangalore, Smith and Veldkamp, 2018). Salinity intrusion is expected to affect 71 percent of the Mekong River Delta and the Red River Delta, the predominant rice growing regions of Viet Nam, along with other coastal

areas. Among crop plants, rice is the most sensitive to salinity. The tolerance threshold for most varieties is 3 deciSiemens (dS) per metre electrical conductivity (Hoang *et al.*, 2016). Rice yields can be reduced by up to 50 percent when grown under moderate salinity levels of about 6 dS per metre.

Induced genetic variation and mutation breeding for crop improvement in Viet Nam

Evolution is driven by spontaneous changes in the DNA of an organism, which are called mutations. These changes are introduced into the genome by a variety of external factors, including different types of radiation and chemicals. Such spontaneous mutations, together with chromosomal crossovers, have been the bases of crop domestication and crop breeding over centuries. Changes can also be induced in the DNA and genome of plant seeds by artificial exposure to mutagen sources such as gamma rays, X-rays, UV rays, chemical mutagens etc. Such induced changes or mutations facilitate a more rapid evolutionary process, facilitating the development of new plant populations exhibiting traits of importance to farmers that can then be selected for individuals. These traits can include increased crop yields, tolerance to extreme growing environments such as drought, high temperatures, saline soils, resistance to diseases and pests and the nutritional quality of the grain.

Novel genetic changes generated through induced mutagenesis are utilized in the process of mutation breeding for the development of improved crop varieties. The exposure of seeds or other plant propagules to a physical mutagen, such as ionizing radiation or a chemical mutagen induces novel heritable variations. The seeds that have been exposed to the mutagen, e.g. radiation or chemical, are grown over two or three generations, before the plant population is subjected to selection. Improved plants selected for traits of interest are further advanced through two to three generations of selection for confirmation, and seed generated from the very last generation is tested under different growing environments in the field before the final selection is made. The selected line then moves towards certification as a variety, multiplication and distribution to farmers. The process, called mutation breeding, is a particularly useful plant breeding technique when the desired plant characteristic is not available in the existing gene pool.

The process of plant mutation breeding can be made more efficient through the use of molecular markers, just as in the case of conventional plant breeding. Molecular markers are specific DNA sequences – ranging from a single to several nucleotides – that have been established by fundamental studies to be associated with a particular characteristic. Knowledge of these molecular markers facilitates their use in the selection process, enabling fine precision in screening, and allowing for the screening of a large numbers of plants.

Precise selection processes with the application of molecular markers or with appropriate phenotyping methods, together with a streamlined breeding schedule, are important components of breeding. Induced genetic diversity used in mutation breeding provides an advantage in selection above other types of breeding where existing genetic diversity is transferred from one variety to another.

While mutation breeding as a crop improvement strategy in Viet Nam dates back to the early 1970s, it began in earnest in 1979 with technical support from the Joint Food and Agriculture Organization of the United Nations (FAO)/International Atomic Energy Agency (IAEA) Centre of Nuclear Techniques in Food and Agriculture, Vienna, Austria for the strengthening of institutional and human capacities

Effect of gamma irradiation on plant height in rice. The picture shows six mutant plants with different heights in the M2 generation (the progeny of plants grown from irradiated seeds)

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in the technology. Five national technical cooperation projects were awarded to the country in 1997, 1999, 2005 and 2014 in addition to the country's participation in seven regional projects between 1999 and 2022. This sustained support over decades enabled the institutionalization of mutation breeding as a crop improvement technique of choice, in particular for rice and soybean.

By the end of 2020, 80 improved mutant crop varieties, including 54 rice and 16 soybean, were registered and officially released in the country. Information on rice and soybean mutant varieties as reported to the FAO/IAEA Mutant Variety Database (http://mvd.iaea.org) is captured in Tables 1 and 2, respectively. While the tables do not capture the full number of released mutant varieties in the country, they provide a good glimpse of the intensity of mutation breeding, frequency of variety release and characteristics of mutant varieties released in Viet Nam. A few varieties not yet in the database are also included in the tables.

The AGI has been at the forefront in the use of mutation breeding for crop improvement in the country. Other institutions that successfully applied the technology in the country are the Institute of Agricultural Sciences for Southern Viet Nam, Soc Trang Department of Agriculture, Food Crop Research Institute, Hanoi Agriculture University and Hanoi Pedagogical University. At the present time, commercial seed companies are leading the development and distribution of new rice varieties. Intellectual property rights apply to all crop varieties developed in Viet Nam since the year 2005. Plant breeders or entities that develop a new variety also retain the right to license or commercialize the variety. Examples of such licensing from the AGI include that of the rice mutant, Khang Dan, to

Screening rice mutant lines for drought tolerance in an experimental field in Daklak, Viet Nam, in 2020 as part of an IAEA-funded project

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the Centural Seed Company in 2007, and of the rice varieties DT 80 and CNC 11 to the Thanh Hoa Seed Company in 2016.

Rice

During the earlier years of the economic reform, i.e. the 1980s, seeds of improved rice varieties were imported into the country for cultivation including CR203 and Jasmine 85 from the International Rice Research Institute, the Philippines; Bac thom 7 (BT 7) from China and Fuji Sakura from Japan (Khanh *et al.*, 2021).

The rice mutation breeding programme in the country released mutant varieties as early as 1975, using as parents introduced varieties such as IR64 and local varieties such as Nang Huong, Tam Xoan, Tai Nguyen and Tep Hanh. While chemical mutagenesis and a combination of irradiation with chemical mutagenesis were used in the early years, irradiation with gamma rays and hybridization using mutant varieties or lines became the main method to develop improved mutant rice varieties since the mid-1990s (Table 1). The mutants VN 124 and VN 24-4 are two examples of varieties derived from crosses between an existing non-mutant variety and a mutant line or variety. For induction of mutation with irradiation, optimal results were achieved by exposing dry or germinated rice seeds to irradiation doses of 200 and 300 Gy in a cobalt-60 (^{60}Co) source at the Nuclear Research Institute, Dalat City in southern Viet Nam. Subsequently, populations of 10 000–15 000 first generation putative mutant (or M1) plants were established and evaluated for desired traits over six generations, i.e. M2 to M7. Following these evaluations, the best lines were approved and

Field testing of mutant rice lines produced by gamma irradiation for resistance to bacterial leaf blight (BLB). The BLB resistant mutant line (right), derived from the Seng Cu variety, showed high resistance to BLB. Photo taken in Hanoi, Viet Nam in 2018
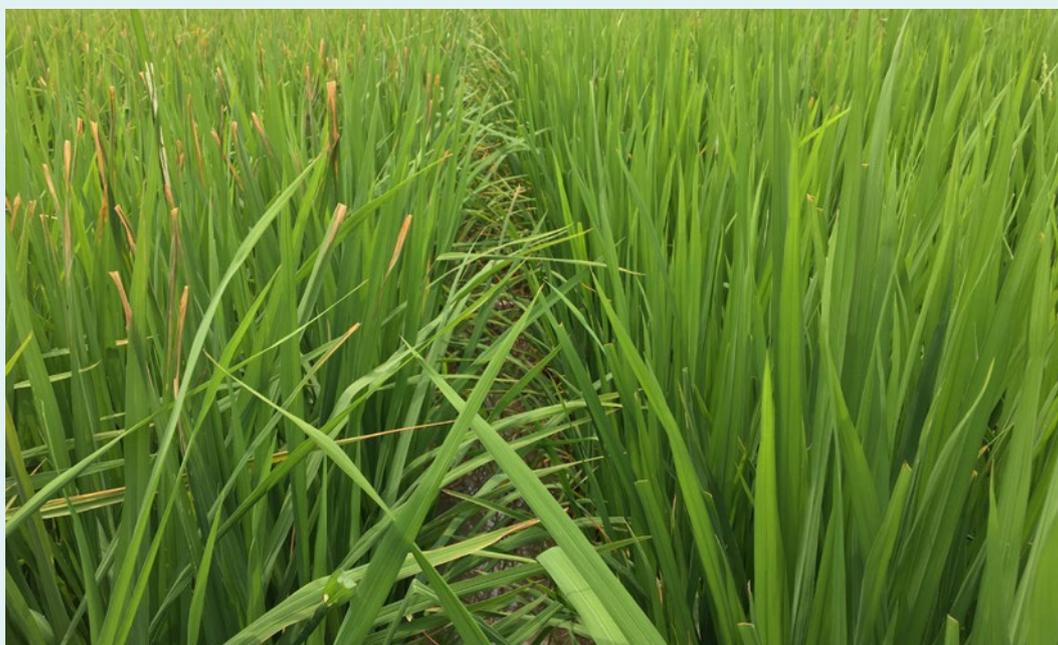
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released for cultivation as temporary or national varieties by the Ministry of Agriculture and Rural Development.

Variety improvement for rice in Viet Nam emphasized productivity initially, and currently focuses on adaptation to climate change and grain quality. The first rice mutant variety bred by AGI, DT 10, was registered in the year 1989 at a time when the country was importing rice, and the average yield of rice in Viet Nam was 3.3 tonnes per ha. DT 10 had a yield of 6 to 7 tonnes per ha and it became a long-standing variety cultivated over a cumulative area of 2.4 million hectares from 1990 to 2014. About 20 percent of the dedicated area under rice in north Viet Nam was grown under this variety.

Promising rice mutant varieties released and grown extensively in the Mekong River Delta include TNDB 100, VND 95-19, VND 99-3, OM 2717 and OM 2718. The variety VND 95-20, released in 1999 from mutagenesis of the imported variety IR 64, has wide adaptation, is tolerant to the acidic soils in the Mekong River Delta, and has a short duration of 90–100 days. VND 95-19, another mutant from IR 64, has a yield potential of up to 10 tonnes per hectare and is resistant to the insect pest, brown planthopper, and the disease, rice blast. The high chalkiness of this variety has restricted it to domestic consumption. VND 99-3, registered locally in 2004 and released nationally in 2006, is an induced mutant from a local aromatic variety, Nang Huong, and is tolerant to the acid sulphate soils and drought conditions of southern Viet Nam. It also has inherited tolerance to iron toxicity from the parent.

Mutation breeding of rice for hull colour and associated nutrient content using induction with gamma irradiation. The mutant line carries both purple and yellow hull seed colours in the same culm. Photo taken in Hanoi, Viet Nam in 2021

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Agronomic screening of mutant rice lines in field experiments in Hanoi, Viet Nam in 2019. The picture shows a mutant line with better plant architecture, taller plants and longer duration

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More recently, the rice varieties DT 80 and CNC 11 were released for cultivation in 2019. DT 80 is a mutant variety developed from the line TL 6.2 which carries the *Saltol* quantitative trait locus (QTL) for salinity tolerance. In addition to salinity tolerance, DT 80 has an average yield of 6 to 7 tonnes per hectare, short duration and good grain quality. In Viet Nam, salinity tolerance was originally bred into the popular variety BT 7 from the introgression line FL478 which in turn inherited the QTL from Pokkali rice, the highly salinity tolerant rice from South India. The simple sequence repeat markers, RM493 and RM3412b, were used to successfully transfer the *Saltol* locus from FL478 into BT 7 in three backcrosses (Linh *et al.*, 2012). CNC 11, also released in 2019, was developed from mutagenesis of BT 7, and has an average yield of 6 to 6.5 tonnes per hectare.

Several aromatic rice mutant varieties, Red ST 3, ST 16, ST 19, ST 20, ST 22 and Purple ST are also popular, and Red ST 3 is known to have a high iron content. These have not yet been reported to the FAO/IAEA Mutant Variety Database. ST 19 and ST 20 are valued in the export market with prices up to USD 900 per tonne, which are about USD 350 per tonne higher than the price of other aromatic rice varieties.

Soybean

Induced mutagenesis was used for the genetic improvement of local and introduced varieties such as Coc chum, DH 4, AK 04 and V 74, and hybrid lines such as D.3/033, D.98-099 and 2001 HC (Le and Pham, 2021). Dry seeds were irradiated with gamma rays using a ^{60}Co source or, in a few instances, were subjected to chemical mutagenesis. Single seed descent and pedigree methods were used for screening the putative mutants from M2 to M5 generations, followed by field evaluations, adaptation testing, variety registration and release.

To date, i.e. October 2022, 16 mutant soybean varieties have been released for cultivation, of which 13 were developed by AGI. These include seven national varieties, namely M 103, DT 83, DT 84, DT 55 (AK 06), DT 90, DT 22 and DT 2008; three regional varieties, namely S 31, DT 95 and DT 99: and a few outstanding lines or varieties including DT 2008 DB (Table 2). These improved soybean mutant varieties are characterized by wide adaptability, high yield and short duration. Their average yield is in the range of 1.8 to 3.6 tonnes per hectare which is 0.8 to 1.5 tonnes per hectare higher than earlier local varieties. The growth duration of 70–100 days enabled three crops per year. In general, these varieties also have a high protein content of 40 to 47 percent.

The black seeded mutant developed from DT 2008 and named DT 215 is the only black seeded variety in the country and has an average yield of 3.5 tonnes per hectare. The long duration of this variety made its incorporation into many cropping systems in the country difficult. Understanding

Soybean mutant variety DT 2008 during the vegetative stage in a farmer's field in the summer-autumn crop. The variety has high productivity and disease tolerance. Photo taken in Ha Giang, Viet Nam, in 2010

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Soybean mutant variety DT 2008 near the harvest stage in a farmer's field in the summer–autumn crop. The high yielding variety is superior to the local variety. Photo taken in Cao Bang, Viet Nam in 2017

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the genetic basis of the black seed colour can facilitate marker-assisted introgression of the trait into short duration soybean varieties. While at least five classical genetic loci are known to control black seed coat in soybean, the genes responsible for seed color are not fully understood. A newly identified black soybean mutant line, DT 26 BS, is indistinguishable from its yellow-seeded parent, DT 26, in all attributes except seed colour. In a coordinated research project supported by the Joint FAO/IAEA Centre, an exploration is in progress to develop a mapping population involving DT 26 and DT 26 BS for the purpose of using the F2 population in a genomic study to determine the causative mutation(s) responsible for black seed colour.

The impact

The adoption and cultivation of induced mutant crop varieties, especially rice and soybean, have contributed to the economic development of Viet Nam.

Rice

Mutant rice varieties became increasingly grown in the Mekong River Delta only after 1995. By the end of the first decade of the twenty-first century, mutant varieties occupied 10.3 percent of the area under modern rice varieties in southern Viet Nam, and the variety VND 95-20 became one of the top five export rice varieties of southern Viet Nam. Prominent mutant varieties of the region, VND 95-20, VND 99-3, TNDB 100, VND 95-19, OM 2717 and OM 2718, returned respectively

Soybean mutant variety DT 2008 during the vegetative stage. The variety has good disease tolerance and high yield potential with higher number of fruits per plant than the control check variety. Photo taken in Son La, Viet Nam in 2015

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Researchers and farmers visit a field with soybean variety DT 2008 in spring crop on a terraced field. The variety has good drought tolerance and is suitable for planting in rice paddy fields with insufficient water. Photo taken in Ha Giang, Viet Nam, in 2010

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USD 300, 9, 37.5, 6, 12 and 8.5 million by 2008 (Do, 2009). The variety VND 95-20 was cultivated on an average annual area of 280 000 to 350 000 hectares since 2000, and during the peak period of its popularity between 2000 and 2007, provided a return equivalent to USD 300 million.

In the northern provinces, the mutant varieties, including CM 1, DT 10, DT 11 and Khang Dan, occupied 40 percent of the cultivated area under rice in 2007, covering about 0.4 million hectares annually (Vinh *et al.*, 2009). The rice mutant variety, DT 10, developed by AGI enjoyed a long period of popularity and was cultivated by farmers for roughly 25 years between 1990 and 2014. During this period, it recorded a cumulative area of 2.4 million ha benefiting 3 million farmers and contributing to an increased income of USD 450 million, calculated based on farm gate value of additional income of roughly USD 188 per hectare. It has an average yield of 6–8 tonnes/ha and at the time of its release in the early 1990s the average productivity of rice was very low at 3.3 tonnes/ha.

The peak annual area under the rice mutant, Khang Dan, released in 2008, was 200 000 ha and it became one of the most popular varieties in north and central Viet Nam providing an increased income of USD 143.8 million for farmers, from calculations based on cumulative area during the cultivation period, yield difference relative to parent variety, and farm gate value of additional produce. The saline tolerant DT 80, identified with marker-assisted selection for the presence of the *Saltol* QTL, can withstand saline soils, and has a cumulative area of 15 000 ha since its release in 2019. Its yield of 6–7 tonnes per hectare provides an average yield advantage of 1–1.5 tonnes per hectare over its parent TL6.2, contributing to increased earnings of USD 330 per hectare annually.

Soybean

For soybean, it is estimated that 60 to 70 percent of the current area under cultivation in the country is planted to mutant varieties. High-yielding, short duration varieties are sought, with the mutant variety DT 2008 cultivated by about 1.5 million farmers. DT 84, released in 1994 and one of the earliest soybean varieties developed in the country, covered 40 percent of the total area under soybean in Viet Nam in 2017. During the period from 2000 to 2019, the cumulative area under the mutant varieties DT 99, DT 2001, DT 96 and DT 2010 was an estimated 156 000 hectares. The yield advantage of these varieties over their parents ranged from 0.2 to 0.8 tonnes, providing a total yield advantage of 76 600 tonnes cumulatively over their years of cultivation, which in turn translates to an estimated value of USD 58.6 million. The mutant variety DT 2008 had the most yield advantage (0.8 tonnes per hectare) and was cultivated over a cumulative area of 30 000 hectares from 2011 to 2019. The popular variety, DT 96, with a yield advantage of 0.6 tonnes per hectare, was cultivated over 60 000 hectares from 2004 to 2019.

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Table 1. Released mutant rice varieties in Viet Nam reported in the FAO/IAEA Mutant Variety Database (as on 1 December 2022)

Name of Mutant Variety	Registration Year	Parent Variety or Line	Method of Variety Development	Characteristics of Improved Variety
VN 10	1975	Rumani 45	Hybridization with mutant induced by irradiation with gamma rays (100 Gy)	N.A.
VN 20	1975	Rumani 45	Hybridization with mutant induced by irradiation with gamma rays (100 Gy)	N.A.
VN 4	1975	C 2-13	Hybridization with mutant induced by irradiation with gamma rays (200 Gy)	Early maturity (90–95 days)
NN 22-98	1983	IR 22	Chemical mutagenesis with 0.02% NEU	Strong culm, adaptation to deep water, higher thousand grain weight, better grain quality, high yield
6 B	1986	IR 42 and Atomita 2	Hybridization of IR 42 with the mutant variety Atomita 2 developed by irradiation with gamma rays (200 Gy)	High yield (20%), larger panicle, higher thousand grain weight, better than both parents in salinity tolerance
DB 250	1987	F1 (TB 1 x IR 22)	Seed of F1 of hybrid irradiated with gamma rays (250 Gy) and treated with chemical mutagen MNU (0.02%)	Resistance to deep water (40–60 cm), resistance to lodging, resistance to bacterial blight and *Pyricularia oryzae*
DB 2	1987	Nep Hoa Vang	Chemical mutagenesis	Early maturity, insensitivity to photoperiod, resistance to low temperature, resistance to diseases
DCM 1	1988	Cuom	Chemical mutagenesis with MNU (0.02%)	Semi-dwarfness, early maturity
MT 4	1988	Moc Tuyen	Chemical mutagenesis with NEU (0.02%)	High resistance to lodging, resistance to environmental stress
DT 10	1989	C 4-63	Combined treatment with gamma rays (200 Gy) and chemical mutagen MNU (0.025%)	High yield, resistance to lodging, resistance to bacterial leaf blight, resistance to insects
A 20	1990	N 20 x N 30	Hybridization of two mutants induced by chemical mutagenesis with MNU (0.015%)	Early maturity, high tolerance to salinity

Name of Mutant Variety	Registration Year	Parent Variety or Line	Method of Variety Development	Characteristics of Improved Variety
MT 6	1993	IR 8 x X 6	Chemical mutagenesis of hybrid seeds of the cross (IR8 x X6) with DMS (0.02%).	Stiff stem, altered leaf morphology (erect leaves)
CM 1	1994	Chiembau	Irradiation with gamma rays (200 Gy)	High yield, good quality
DT 11	1994	C 4-63	Combined treatment with gamma rays (200 Gy) and chemical mutagen NEU (0.0025%)	Resistance to diseases, high yield, good quality
DT 33	1994	CR 203	Irradiation with gamma rays (200 Gy)	High yield, good quality
VND 95-26	1995	IR 9729	Irradiation with gamma rays (200 Gy)	Early maturity, good grain quality, high yield
TNDB 100	1997	Tai Nguyen Duc	Irradiation with gamma rays (50 Gy)	Shorter plant height, early maturity, resistance to diseases, resistance to BPHA, grain quality, adaptability
CM 6	1999	Chiembau	Irradiation with gamma rays (200 Gy)	High yield, good quality
THDB	1999	Tep Hanh	Irradiation with gamma rays (200 Gy)	Shorter plant height, early maturity, resistance to diseases, resistance to brown planthopper, grain quality, adaptability
VND 95-19	1999	IR 64	Irradiation with gamma rays (200 Gy)	Strong tolerance to acid sulphate soil, sturdy stem, high yield (5–10 t/ha), resistance to brown planthopper and blast disease. Good parent in rice improvement program. Suitable for cultivation in adverse conditions.
VND 95-20	1999	IR 64	Irradiation with gamma rays (200 Gy)	Short duration (90–95 days), stiff stem, high yield (6–9 T/ha), good quality (long grain, amylose content = 20–22%), wide adaptation (tolerant to acid sulphate soil, salinity, rainy season), intermediate resistant to brown planthopper, blast disease, some main pests

Name of Mutant Variety	Registration Year	Parent Variety or Line	Method of Variety Development	Characteristics of Improved Variety
Mutant Tam thom	2000	Tam thom	Irradiation with gamma rays (100–200 Gy)	High yield
DT 22	2002	[DV 2/Nep 415] F1	Chemical treatment of hybrid seeds from F1 generation	High yield
DT 36	2002	[VN 01/BG 90-2] F6	Irradiation of *in vitro* culture with gamma rays (50 Gy)	High grain yield, good grain quality, short growth duration, photoperiod insensitivity, tolerance to lodging and environmental stresses, resistance to pests and diseases
Mutant Khang dan	2003	Khangdan	Irradiation with gamma rays (100 Gy)	High yield, tolerance to pest and disease
DT 21	2004	DV 2	Induced mutation combined with hybridization	Glutinous rice, short growth duration allowing 2 crops per year, average yield 4.0–4.5 tonnes per ha, good quality, soft and scented cooked rice, suitable for consumers and for export
OM 2717	2004	TNDB 100 and Tai Nguyen Duc	Hybridization of Tai Nguyen Duc with mutant variety TNDB 100 obtained by irradiation with gamma rays (50 Gy)	Short duration (90–95 days), high yield (4–7 tonnes per ha), good quality (long grain, amylose content: 20–24%), wide adaptation, intermediate resistance to brown planthopper and blast disease
OM 2718	2004	OM 1738	Irradiation with gamma rays (50 Gy)	Short duration (90–95 days), high yield (5–8 t/ha), good quality, wide adaptation, intermediate resistance to brown planthopper and blast disease
VND 99-3	2004	Nang Huong	Irradiation with gamma rays (200 Gy)	Short duration (90–100 days), stiff stem, high yield (5–8 t/ha), good quality, tolerant to adverse conditions (acid sulphate soil, salinity), resistant to brown planthopper, blast disease, tolerant to grassy stunt virus disease
DT 38	2007	KD 18	Irradiation with gamma rays (200 Gy)	Higher grain yield, non-lodging, good resistance to some main pests and diseases

Name of Mutant Variety	Registration Year	Parent Variety or Line	Method of Variety Development	Characteristics of Improved Variety
Mutant Khang Dan	2008	Khang Dan		High yield, tolerance to pest and disease
DB 5	2008	28 R	N.A.	High yield, tolerance to pest and disease
DB 6	2008	28 R	N.A.	High yield, tolerance to pest and disease
VN 121	2008	VND 31, VND 22-26, Tam Xoan	Hybridization of female line VND 31, a mutant derived from Tam Xoan, with male line VND 22-26 also a mutant line	Better plant type, high yield, good quality (aroma, long grain, no chalkiness) and tolerant to brown planthopper, blast disease, tolerant to grassy stunt virus disease
VN 124	2008	VND 22-62	Hybridization of female line, Qimiaxiang, with mutant male line, VND 22-62	Short duration, aroma, good quality for export, tolerant to brown planthopper, blast disease and grassy stunt virus disease
VN 24-4	2009	IR 64, VND 95-19	Hybridization of female variety IR 64 and male mutant variety VND 95-19	Bigger panicles, stiff culms, strongly seedling vigour, high tolerance to brown planthopper, grassy stunt virus and adverse conditions
PD 2	2010	Khau Mang	N.A.	Glutinous aromatic grain, photosensitive, high yield
Mutant P 6	2011	P 6	N.A.	Short duration (85–90 days) in summer season, tolerant to high temperature
Nam Dinh 5	2012	Tam thom	N.A.	Grain quality, aromatic grain, high yield
DT 39 Quelam	2013	Bac Thom	N.A.	Grain quality, high protein, high yield, resistance to leaf blight
DT 80	2019	TL 6.2	Irradiation with gamma rays (250 Gy)	Salt tolerance, high yield, short duration and good quality
CNC 11	2019	BT 7	Irradiation with gamma rays	Salt tolerance, high yield of 6 to 6.5 tonnes per hectare

Gy: Gray; NEU: N-nitroso-N-ethylurea; MNU: N-methyl-N-nitrosourea; DMS: Dimethyl sulphate;
N.A.: Information not available

Table 2. Released mutant soybean varieties in Viet Nam

Name of Mutant Variety	Registration Year	Parent Variety or Line	Method of Variety Development	Characteristics of Improved Variety
DT 83	1987	Coc chum	Chemical mutagenesis with 0.04% EI	Yellow seed colour and high yield
DT 90	1993	[G7002 x Coc chum] F4	F4 seeds of hybrid [G7002 x Cocchum] irradiated with gamma rays (180 Gy)	High yield and high protein content
DT 84	1994	D.3/033-F3 [DT 80 x DH 4]	F3 seeds of hybrid [DT 80 x DH 4] irradiated with gamma rays (180 Gy)	High yield
M 103	1994	DH 4	Combined treatment of seeds with gamma rays (50 Gy) and the chemical mutagen, EI (0.01%)	High grain yield, resistance to lodging and high grain quality
S 31	1995	V 74	Combined treatment with of seeds with gamma rays (180 Gy) and the chemical mutagen, EI (0.04%)	High yield and tolerance to low temperature
DT 95	1998	AK 04	Irradiation with gamma rays (150 Gy)	Early maturity, high yield and yellow seeds
DT 99	2000	D.98-099-F4 [IS 01 x Cuc]	F4 seeds of hybrid [IS01 x Cuc] irradiated with gamma rays (150 Gy)	Early maturity, good adaptability, resistance to diseases and tolerance to high and low temperatures
DT 2001	2001	N.A.	N.A.	N.A.
DT 55 (AK 06)	2002	DT 74	Irradiation with gamma rays (100 Gy)	Early maturity and high yield
DT 96	2004	DT 84 x DT 90	Hybridization of mutant variety DT 84 with non-mutant DT 90	Moderate tolerance to drought, rust resistance, wide adaptability, growth duration: 88–100 days, suitable for raising 3 crops per year, yield: 1.8–3.6 tons/ha, high protein content (43–45%)
DT 22	2006	DT 12 x DT 95	Hybridization involving mutant line	Early maturity
DT 2008	2010	2001HC-F4 [DT 2001 x HC 100]	F4 seeds of hybrid [DT 2001 x HC 100] irradiated with gamma rays (180 Gy)	High yield, resistance to rust, downy mildew, tolerance to drought

Name of Mutant Variety	Registration Year	Parent Variety or Line	Method of Variety Development	Characteristics of Improved Variety
DT 2008 DB	2015	DT 2008	N.A.	Seed colour yellow to black; higher content of carotenoids, omega 3 and omega 6 (58, 30 and 12 %, respectively), early maturity (by 5–8 days)
DT 96 DB	2015	DT 96	N.A.	Resistant to lodging
DT 26 DB	2015	DT 26	N.A.	Seed colour yellow to black
DT 2010	2017	DT 2008 x DT 99	Hybridization of two mutant varieties: DT 2008 x DT 99	Early maturity (80–88 days), high yields (1,8–2,5 tonne/ha, resistance to rust, tolerance to drought

Gy: Gray; EI: Ethylenimine; N.A.: Information not available
Information extracted from the FAO/IAEA Mutant Variety Database (1 December 2022) and complemented with varieties reported by Le and Pham (2021).

3.3 Bt eggplant in Bangladesh: improving food security and farmer incomes through genetic engineering

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Abstract

The major constraint to the production of eggplant (brinjal) is the persistent and widespread infestation by an insect called the eggplant fruit and shoot borer (EFSB). Even with the unsustainably high use of insecticides to manage EFSB, farmers would lose 30 percent to 60 percent of the crop to the pest. Bt eggplant, which provided effective control of the EFSB, was developed by the Bangladesh Agricultural Research Institute (BARI) using the Cry1Ac gene, which was provided by Mahyco, India. The four Bt varieties developed and commercialized by BARI – BARI Bt Begun-1 (Uttara), BARI Bt Begun-2 (Nayantara), BARI Bt Begun-3 (Kazla) and BARI Bt Begun-4 (ISD006) – were widely adopted by farmers. Bt eggplant therefore became the first genetically modified vegetable crop in the world to be developed for the benefit of smallholder farmers in developing countries. In 2013, Bangladesh became the first country to approve Bt eggplant for commercial cultivation. The adoption of Bt eggplant offered an avenue for farmers to improve farm productivity and the quality of the produce while significantly reducing insecticide use, improving food security and nutrition and enhancing the livelihoods of the mostly smallholder farmers who grow the crop. Farmers who planted Bt eggplant saved 61 percent on pesticide costs and earned a net return of USD 2 151/ha, compared to USD 357/ha earned by those who cultivated non-Bt eggplant, a six-fold difference. The adoption of the technology therefore resulted in significantly reduced input costs, enhanced the market value of the produce while also mitigating the major health and environmental concerns posed by eggplant cultivation that is enabled by the use of inordinate amounts of pesticides in Bangladesh.

The problem

Eggplant (*Solanum melongena* L.), known as brinjal (begun) in Bangladesh, is the second most important vegetable grown in the country after potatoes and is particularly valued for its the vitamins, minerals, fibre and antioxidant richness that it contains. A single serving of eggplant provides at least 5 percent of a person's daily requirement of fibre, copper, manganese, vitamin B-6 and thiamine. In the 2020–21 growing reason, resource-poor farmers cultivated the crop on about 83 000 and 49 000 acres in winter and summer respectively. More than 100 varieties of eggplant are grown across almost all agro-climatic zones of the country, offering fruits of varied colours, sizes, shapes and flavours.

For farmers growing eggplant in Bangladesh, productivity is severely constrained by continual and extensive infestation by the eggplant fruit and shoot borer (EFSB), *Leucinodes orbonalis Guenée* (Lepidoptera: Crambidae). In addition to damaging the eggplant shoots and flowers, the EFSB

The eggplant fruit and shoot borer causes severe damage to traditional eggplant varieties. Azharul Ali Mondol shows fruit damaged by the insect in a field growing non-Bt eggplant in Gaibandha, Bangladesh in February 2023

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caterpillars severely damage the fruit by boring into it, thereby reducing its market value. To control the pest, farmers spray broad-spectrum insecticides up to 100 times (Shelton *et al.*, 2018) during the growing season. This practice accounts for 35 to 40 percent of the crop's production cost and poses serious risks to the farm economics for small and marginal farmers. Despite the high use of pesticides, farmers still face yield losses of 30 to 60 percent due to EFSB infestation (Shelton *et al.*, 2018). The excessive application of insecticides also leads to high pesticide residue levels on the fruit, which is harmful to human health and causes environmental concerns.

The solution

Product development

The India-based Maharashtra Hybrid Seed Company (Mahyco) used the insect resistance Cry1Ac gene from *Bacillus thuringiensis* (Bt) to transform eggplant in the early 2000s. The resultant genetically engineered Bt eggplant (event "EE-1") demonstrated excellent levels of resistance to EFSB under field conditions (Shelton *et al.*, 2019). The Bt Cry protein, which has been used extensively in Bt cotton and Bt corn and as a component of many organic biopesticides, has a long history of safe use (Center for Environmental Risk Assessment, 2011).

In 2003, the technology (genetically engineered Bt eggplant with EE-1 event) was licensed to the Bangladesh Agricultural Research Institute (BARI), the premier national agricultural research institute of Bangladesh, under the aegis of the Agricultural Biotechnology Support Project II (ABSP II, http://absp2.cornell.edu/), which was funded by the United States Agency for International Development (USAID) (Shelton *et al.*, 2017). Mahyco retains the right to grant licenses to any other parties.

The Cry1Ac gene (EE-1 event) was subsequently introgressed into several locally developed, well-adapted and commercially popular open-pollinated eggplant varieties. These varieties underwent seven years of greenhouse and confined field trials by BARI across various agroclimatic zones of Bangladesh to test its efficacy and environmental safety as per local regulatory requirements. These rigorous agronomic and biosafety trials showed that Bt eggplant was an effective means for controlling EFSB without using pesticides. Following ten years of research and development activities, four varieties of Bt eggplant (BARI Bt Begun-1, BARI Bt Begun-2, BARI Bt Begun-3 and BARI Bt Begun-4) were approved for commercialization on 29 October 2013. This made Bangladesh the first South Asian country to approve the commercial cultivation of a genetically engineered crop (Shelton *et al.*, 2019). Following the approval, in January 2014, by the Ministry of Agriculture, Bangladesh, the seeds of the four varieties were supplied to 20 farmers for cultivation. As part of insect resistant management studies and for the long-term sustainability of Bt brinjal in Bangladesh, studies evaluating the susceptibility of insects were conducted to monitor natural variation in susceptibility to Cry1Ac for future comparisons when Bt brinjal is more widely adopted (Prodhan *et al.*, 2019).

Product dissemination

Upon the closure of the ABSP II project in 2015, a follow-up cooperative agreement was entered between USAID and Cornell University under the auspices of the Feed the Future South Asia Eggplant Improvement Partnership (SAEIP) to facilitate the enhanced adoption of Bt eggplant in Bangladesh. The SAEIP project's focus was on building institutional and human capacity across the supply chain, promoting responsible product stewardship, monitoring and evaluating the post-release impact, and effective communication to help scale up the adoption of Bt eggplant.

The successful dissemination of Bt eggplant in Bangladesh was made possible by the government's leveraging of three key public sector institutes to scale up the adoption of the varieties by eggplant farmers in the country: (a) BARI; (b) the Department of Agricultural Extension (DAE), with a mandate for disseminating technologies and production practices to farmers; and (c) Bangladesh Agricultural Development Corporation (BADC), the leading public sector seed production and distribution agency. With overall project management and technical-commercial support, such as carrying out field trials, technology demonstrations, seed production and seed quality testing, from Cornell University and Sathguru Management Consultants, these three public sector institutes in Bangladesh worked collaboratively towards the common goal of sustained diffusion of Bt eggplant varieties in Bangladesh (Figure 1).

BARI conducted farmer field demonstration trials with the new Bt eggplant varieties across various districts through its On-Farm Research Division, thereby gradually increasing the farmer number and acreage over the first three seasons. BADC handled seed bulking, while DAE undertook the wider distribution and promotion of these newly improved varieties (Figure 2).

The strengthening of the stakeholder capacities was critical for the sustained adoption of Bt eggplant in the country. BARI led the continual sensitization of farmers and officials of DAE and BADC on various aspects of Bt eggplant production, management and stewardship, i.e., "the legal, ethical and moral obligation to assess products and technologies to ensure that they are safe as well as socially and environmentally responsible" (Mbabazi *et al.*, 2021) to ensure the appropriateness of the information being disseminated to the target farmers. In this regard, several farmer field days were conducted by BARI across different districts to demonstrate the improved traits of the new varieties and their production practices to sensitize the farmers to the benefits of Bt eggplant. By

Figure 1. Project partners and their roles

Project Lead and Regional Coordination Cornell University, United States of America Sathguru Management Consultants, India	• Overall technical guidance and progress monitoring • Work with all the stakeholders (BARI, BADC, DAE, farmers, MoA) in Bangladesh
Technology Provider (Licensor) Mahyco, India	• Developed the event (EE-1 with Cry 1 Ac gene) to confer resistance to EFSB • Biosafety and regulatory studies • Introgression of EE-1 event into Bangladeshi varieties and back cross-breeding for best-performing line selection • Technical training and advisory to the product development team
Product Developer and Implementing Partner (Sub Licensee) Bangladesh Agricultural Research institute (BARI)	• Non-exclusive, pro bono access to genetically engineered (GE) Bt eggplant, development of Bt eggplant varieties: breeding, line selection, development and commercialization of transgenic lines • Nucleus, breeder, foundation seed production, processing, packing, labelling • Seed quality testing for seeds produced by BARI and BADC • Large-scale field demonstration trials in farmer fields • Capacity building of stakeholders (extension agents, seed producers, marketing officers, dealers, farmers)
Seed Production Entity (Public) Bangladesh Agricultural Development Corporation (BADC)	• Foundation seed (FS) production by sourcing breeder seed from BARI • Seed production, packaging and labelling as per GoB guidelines, refuge packet inclusion in Bt eggplant seed packets • Seed sale through dealers to farmers and nurseries • Farmer orientation
Extension Entity (Public) Department of Agricultural Extension (DAE)	• Extension services • Product promotion • Farmer training
Beneficiaries Farmers	• Demonstration trials • Cultivation

the end of the SAEIP in 2021, Bt eggplant seeds had been delivered to over 60 000 farmers in the country.

Through the SAEIP project, a collaboration between these three organizations on the production, distribution and adoption of Bt eggplant in Bangladesh was facilitated. The project was also used for sensitizing the farmers, other stakeholders, such as agriculture extension agents, BADC marketing officers, dealers and the general public on crop varieties arising from genetic modification and their management for strengthening the capacities of stakeholders.

Azharul Ali Mondol, a farmer in the village of Saigari Gobindapur, Gaibandha, holds Bt eggplant harvested from his field in February 2023. Mondol first started cultivating Bt eggplant in the 2022–23 season after receiving seeds from the BARI's On-Field Research Division Station in Alamnagar

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Key factors that contributed to the success of the initiative

For the first time, the public sector in Bangladesh was involved in successfully developing and commercializing a genetically engineered food crop. The unique public–private partnership model was the key driving force for the success of the technology. This demonstrated political will by the government in creating the enabling environment both for R&D activities to be conducted and for the outputs to be disseminated. There was an identified constraint for which a solution had to be found, and the government led the way. Significantly, the partnerships enabled access to proprietary technologies and the provision of long-term funding. The identification and assignment of complementary responsibilities to national institutions was also critical to the success. The promotion of effective laboratory and field stewardship practices – including trait performance, resistance management, integrated pest management, good agricultural practices, maintaining high-quality seeds and planting material – and thorough in-season monitoring of demonstration plots and farmers' fields led to the successful adoption of the technology.

The significant efforts invested in involving farmers in the trialling of the new varieties made them co-creators of the solution and therefore enhanced the adoption of the new varieties. The strengthening of the capacities of stakeholders on aspects related to seed quality testing, quality seed dissemination, stewardship guidance for farmers and Bt eggplant cultivation and management practices for farmers were also critical success factors. Equally critical was the creation of public awareness regarding the safety and utility of Bt eggplants in the country. In this regard, farmer sensitization efforts and the promotion of science-based public awareness of biotech crops further contributed to the success.

Figure 2. Timeline for the commercialization and adoption of Bt eggplant varieties in Bangladesh

Four varieties of Bt eggplan- BARI Bt Begun 1, BARI Bt Begun 2, BARI Bt Begun 3 and BARI Bt Begun 4 were approved for cultivation in Bangladesh	BARI conducted **1 900** demostration trials and farmer trainings	BADC started producing Bt eggplant seeds for commercial distribution via their outlets	Synchronized scaling-up through collaboration among organizations

October 2013	January 2014	2014 onwards	2016 onwards	2017 onwards	2018 onwards	2019	2021
	MoA, Bangladesh distributed Bt eggplant seedlings to **20 farmers** across 4 districts		DAE started distribution of BARI's seeds for wider adoption		Coordinated and focused efforts by BARI, BADC & DAE for adoption of stewardship practices (genetic purity, protein expression, refuge planting, etc.)		**>65 000 eggplant farmers** cultivating Bt eggplant across **64 districts** (include farmer-saved seeds)

MoA (Ministry of Agriculture), DAE (Department of Agricultural Extension), BADC (Bangladesh Agricultural Development Corporation), BARI (Bangladesh Agricultura Research institute)

The challenges were multi-fold and included: the absence of a single regulatory entity with the necessary experience; potential risks of poor performance of the varieties across farmer fields; lack of farmers' understanding of the management of biotech crops; and limited science-based public awareness about the genetically modified crop varieties. Furthermore, the wider adoption of the Bt eggplant varieties in Bangladesh was limited by the availability of only four varieties which were not suitable for all the crop's growing areas of the country. Additionally, many eggplant farmers did not know the cultivation practices for Bt eggplant and therefore needed a more elaborate extension programme, which was beyond the scope of the SAEIP project. The open pollination reproduction of the varieties, seed saving by farmers and informal seed multiplication and distribution have further complicated the stewardship efforts. Presently, only one technology developer (BARI) is involved in the commercialization or seed distribution process of these new varieties. No private sector entity participates in the seed value chain.

Farmer impact

The adoption of Bt eggplants resulted in the reduced application of pesticides, which translates to significant economic and health benefits to farmers while reducing the environmental footprint of the cultivation of the crop. Bt brinjal provided an average 19.6 percent higher yield and 21.7 percent higher revenue than non-Bt varieties (Shelton *et al.*, 2020). In a study conducted by BARI scientists in 2017, a six-fold difference was observed in the farmers' net returns/ha (USD 2151/ha for Bt brinjal as compared to USD 357/ha for non-Bt brinjal). The study also identified that farmers spent 61 percent less on pesticides compared to non-Bt brinjal farmers and experienced no yield losses due to the EFSB (Rashid, Hasan and Matin, 2018).

The rapid adoption of the Bt eggplant in Bangladesh by more than 65 000 farmers in seven years indicates the benefits the farmers derive from the technology. An estimated 18 percent of the winter eggplant area is under Bt eggplant cultivation (based on seeds distributed) as of the 2020-21 growing season. Additionally, an estimated 15 to 20 percent of farmers save their Bt eggplant

Figure 3. Economic and health benefits derived by Bt eggplant growing farmers. The estimates come from Prodhan *et al*. (2018), Rashid, Hasan and Matin (2018), Ahmed *et al*. (2019) and Shelton *et al*. (2020)

Negligible fruit infestations in Bt eggplant compared to **45%** in the non-Bt eggplant

61% saving on pesticide cost to farmers as compared to non-Bt eggplant farmers

19.6% higher fruit yield in Bt eggplant against non-Bt eggplant

6X increase in farmers net returns to USD 2151/ha for Bt eggplant/ha as compared to USD 357/ha for non-Bt eggplant

21% increase in gross revenue

41% reduction in toxicity of pesticides applied, measured by Pesticide Use Toxicity Score (PUTS)

seeds from the previous season, indicating an even more significant number of adopters benefiting from the technology. According to several studies, the product has led to a multi-fold impact on the farmers' lives, as indicated in Figure 3.

Parul Begum and Ismail Mondol harvest Bt eggplant from their field in Mission Bongobandhu Para, Khajanpur, Joypurhat Sadar in February 2023

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Liton Mia harvests Bt eggplant in a field in Thengamara, Bogura in February 2023

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Current status of the dissemination of Bt eggplants (as of October 2022) and the way forward to sustain the technology in Bangladesh

Through the USAID-funded Feed the Future Insect-Resistant Eggplant Partnership (IREP, https://bteggplant.cornell.edu/), further efforts are being invested in enhancing the adoption of Bt eggplant technology in Bangladesh. The IREP project focuses primarily on scaling up Bt eggplant adoption through developing and multiplying agronomically superior Bt eggplant varieties, incorporating improved wilt resistance and broader adaptation to agroecological zones, and developing next-generation Bt eggplant hybrids in collaboration with the private sector. Efforts at enhancing the adoption rates also target engagement with vegetable seedling nurseries – to produce seedlings – and various outreach activities with farmers.

Building on the initial success, introducing the Bt gene into newer agronomically superior and consumer-preferred varieties suitable for different regions will help increase the acreage under Bt eggplant cultivation and encourage wide-scale adoption. The long-term sustainability of Bt eggplant in Bangladesh can be strengthened by directing efforts towards developing additional, highly adapted, agronomically superior and marketable varieties. The introduction of second-generation Bt eggplants with dual Bt genes would enhance the durability of the resistance and delay the build-up of resistance in EFSB.

Maricelis Acevedo, Director of the Feed the Future Insect-Resistant Eggplant
Partnership, engages with farmer Parul Begum in her field in Mission
Bongobandhu Para, Khajanpur, Joypurhat Sadar in February 2023

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In Bangladesh, the private sector is growing and dominating the seed supply of major crops, including rice, maize, potato, jute and vegetables. It is now time for the private sector to develop a second-generation product; introduce high-yielding Bt eggplant varieties, including hybrids; increase stewardship compliance and leverage well-established marketing and distribution networks for accelerated and broader adoption. Collaborative efforts between these public sector institutions and private sector entities could enable further popularization of the technology among farmers. The new phase of the project is actively working to address these issues and paving the way for the increased adoption and second generation of Bt eggplant in Bangladesh.

Lessons learned

The four varieties released for commercial cultivation are not agronomically suitable for all eggplant growing areas of Bangladesh and are mostly adapted for the winter growing season. To overcome this, the new phase of the IREP project is developing agronomically superior, wilt tolerant, year-round growing varieties. Bt versions of these new varieties have the potential to increase the adoption and cover more eggplant growing areas in Bangladesh, thereby increasing the impact of the technology. Currently, the Bt eggplant in Bangladesh is being distributed by BARI and DAE to farmers and by BADC at a nominal cost. This has helped a large number of smallholder farmers adopt Bt eggplant cultivation. However, the reach has been limited in part due to supply chain constraints and dealer-

Shohidul Islam, a smallholder farmer in Thengamara, Bogura, showcases some of his Bt eggplant production in February 2023. Farmers in Bangladesh are seeing improved quality of their product by growing Bt eggplant

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level distribution. To enlarge the reach of popular varieties to smallholder farmers, commercial nurseries providing seedlings will create timely access to the planting material, which are leveraged during the new phase of the project.

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3.4 Genomics-assisted breeding delivering superior chickpea varieties in India, Ethiopia, Kenya and the United Republic of Tanzania

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Abstract

The rising number of food-insecure people in the developing world puts enormous pressure on agrifood systems to improve productivity and resilience. Adopting innovative genomic technologies and new breeding methods will help to achieve a faster increase in crop yields. A shift from resource-intensive to low-input agriculture necessitates considerable changes in crop breeding strategies to develop future cultivars. The case study highlights the key achievements in translational genomics of legume crops which are of paramount significance in terms of affordable protein sources and livelihood support to the resource-poor. Legumes are also important to diversify agrifood systems in the developing world. To illustrate the achievements, we provide success stories on genomics-assisted breeding of valuable traits into different genetic backgrounds, resulting in improved crop technologies equipped with biotic and abiotic adaptation and improved quality.

Over the last decade, we developed a plethora of genomic resources, including the whole genome sequences of chickpea, one of the major legume crops. The genome sequencing of germplasm collections has revealed beneficial traits or genes for designing strategies to tailor and develop new cultivars according to evolving agricultural demands. Notable examples include improved varieties with high levels of drought tolerance (Pusa 10 216, Pusa Chickpea 4 005 and IPC L4-14) and Fusarium wilt resistance (Super Annigeri 1, Pusa Chickpea Manav and IPCMB 19-3) in chickpea which were released for commercial cultivation across various agroecologies in India. Similarly, fast-track introgression of drought tolerance facilitated the release of the Geletu and Girar varieties in Ethiopia. Several improved lines developed through genomics-assisted breeding are in advanced stages in varietal release pipelines in several countries including India, Ethiopia, Kenya and the United Republic of Tanzania. We advocate for improved seed production and distribution systems

together with adopting suitable agronomic practices to harness the full potential of these new varieties in farmers' fields. The sustainability and resilience of global crop production also requires a climate-adaptive seed system that relies on rapid deployment of the latest crop varieties in farmers' fields and the timely replacement of older ones.

Introduction

Universal access to sufficient quantities of nutritious food, as envisaged in the Sustainable Development Goals, is fundamental to human well-being. The world population is projected to increase by 2 billion to reach 9.8 billion by 2050 and 11 billion by the end of this century (Adam, 2021). Despite the efforts to improve food security and nutrition, millions of people, especially in sub-Saharan Africa and South Asia, do not have access to sufficient quantities of nutritious food. Due to severe extreme weather events attributed to climate change, significant crop losses have occurred in the dryland tropics, affecting the inhabitants' food security, nutrition and livelihoods. The productivity and resilience of agrifood systems, which are already constrained by climate change and scarce arable lands and water, must be enhanced to reverse the continually rising number of food-insecure and malnourished people, especially in the developing world.

Legume crops, including chickpea (*Cicer arietinum* L.), provide, on average, twice the protein content of cereals, enhance soil fertility through nitrogen fixation and are typically amenable to low-input production systems, thereby making them important for the diversification of agrifood systems, especially in the developing world. Chickpea is a valuable source of many important proteins, minerals and vitamins among legumes and is an important source of protein for a vegetarian diet. Chickpea has one of the most balanced nutritional compositions, and its protein digestibility is the best among the dry season food legumes. Apart from human consumption, chickpea also has economic importance in animal feed and herbal medicine.

As an affordable source of dietary protein, chickpea is critically important for the food security, nutrition and livelihoods of millions of people, especially the resource-poor farmers and their households who grow and subsist on the crop in sub-Saharan Africa and Asia. However, the crop's yield is reduced significantly by drought and two diseases, Ascochyta blight and Fusarium wilt. The crop's breeding programmes have not been effective in developing well-adapted varieties that demonstrate sufficient levels of resistance to these stresses quickly enough to keep pace with the continually evolving stresses.

Traditional breeding efforts have contributed to increasing crop production. However, the rate of enhanced crop production is not enough to meet the food demand of the fast-growing world population. With the availability of large-scale genomic resources and markers associated with the traits of interest, we have the opportunity to accelerate the crop improvement programme by deploying molecular breeding/genomics-assisted breeding (GAB) to enhance the rate of genetic gain. The adoption of GAB, enabled by the availability of genomics resources, demonstrably holds promise for the accelerated genetic improvement of chickpea. The development of genomics resources for chickpea and the use of these in GAB to introgress valuable traits into the crop's different genetic backgrounds rapidly, resulting in improved varieties that have resistances to biotic and abiotic stresses, and which are adopted by farmers in sub-Saharan Africa and South Asia, are described here.

Genome sequencing and the development of markers

Whole-genome sequencing, genome-wide genotyping, genome-wide association studies, the cataloguing of sequence variations – at individual and population levels – and phenotyping are used for the elucidation of the modes of inheritance of the alleles that control traits of interest, such as resistance to various biotic and abiotic stresses. In this regard, recent scientific and technological advances in next-generation sequencing technologies and the significant reduction in costs have augmented the speed, throughput and cost-effectiveness for the generation of digital sequence data, which constitute invaluable genomics resources.

Over the last decade, the chickpea genome was sequenced at the Center of Excellence in Genomics and Systems Biology of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India (Varshney *et al.*, 2013a). This significant undertaking involved the whole genome re-sequencing of a reference set of diverse chickpea genotypes and the sequencing of 3 366 lines from the global composite collection under the auspices of "The 3 000 Chickpea Genome Sequencing Initiative". The annotation of the sequences yielded a total of 205 single nucleotide polymorphisms present in 79 unique genes and the haplotypes of key candidate genes, including those for drought and heat tolerance (Varshney *et al.*, 2019; Varshney *et al.*, 2022). Additionally, a consensus genetic map comprising 352 loci was constructed (Varshney *et al.*, 2014a).

These resources were used to identify the genomic regions, known as quantitative trait loci (QTL), which control drought tolerance and resistance to Ascochyta blight and Fusarium wilt diseases (Table 1), three stresses that impact negatively on chickpea production (Sabbavarapu *et al.*, 2013). Furthermore, markers associated with tolerance to salinity (Soren *et al.*, 2020), heat (Paul *et al.*, 2018), resistance to *Helicoverpa* (Barmukh *et al.*, 2021), and some quality traits (Roorkiwal *et al.*, 2022) were also identified.

Table 1. Key traits mapped and validated for GAB in chickpea

Trait	QTL	Reference
Drought tolerance	QTL-hotspot	Varshney *et al.*, 2014a
Resistance to Ascochyta blight	Ar3 and ar1 & ar2a	Iruela *et al.*, 2006 Iruela *et al.*, 2007 Udupa and Baum, 2003
Resistance to Fusarium wilt	foc 1, 2, 3 & 4	Sharma and Muehlbauer, 2007 Gowda *et al.*, 2009

Use of genomics tools in the genetic improvement of chickpea

Integrating genomics-based tools and techniques in a plant breeding programme accelerates the varietal improvement process while enhancing overall accuracy in decision making and, ultimately, the resultant genetic gains (Figure 1). The GAB methods for using genomics resources in plant breeding include principally marker-assisted backcrossing (MABC), marker-assisted selection (MAS), gene pyramiding, marker-assisted recurrent selection (MARS), forward breeding, haplotype-based breeding (HBB) and genomic selection.

To introgress the target trait using MABC, the recurrent parent (female) is crossed with the donor (male) parent to generate F_1 seeds. Trait-linked markers are used for foreground selection to confirm the presence of the desired trait allele. Genome-wide markers are used as background markers to ensure the recurrent parent genome recovery in the offspring. After two to three rounds of backcrossing and selfing, offspring with desired traits using foreground markers and genome recovery based on background markers are selected.

ICRISAT, in collaboration with partners in sub-Saharan Africa (Ethiopian Institute of Agricultural Research, Ethiopia; Amhara Institute of Agricultural Research, Ethiopia; Egerton University, Kenya; Tanzania Agricultural Research Institute, the United Republic of Tanzania) and Asia (ICAR-Indian Agricultural Research Institute [ICAR-IARI], Delhi; ICAR-Indian Institute of Pulses Research [ICAR-IPR], Kanpur; University of Agricultural Sciences, Raichur and ICAR-All India Coordinated Research Project on Chickpea [AICRP-Chickpea]), implemented GAB for chickpea. The aim was to enhance drought tolerance (Varshney *et al.*, 2013b; Bharadwaj *et al.*, 2021), disease resistance (Varshney *et al.*, 2014b, Pratap *et al.*, 2017; Mannur *et al.*, 2019) in otherwise well-adapted varieties. We also made a recent effort to combine drought tolerance with disease resistance in Pusa 391 as a recurrent parent using MABC (Bharadwaj *et al.*, 2022).

In summary, a total of nine improved chickpea varieties, which were developed through molecular breeding that was made possible by the genomics resources described above, were released in Ethiopia and India between 2019 and 2022 (Table 2).

Figure 1. An integrated crop improvement approach is needed for accelerated varietal improvement. Next generation sequencing-based large-scale genome sequencing of germplasm together with precise phenotyping data is used for the identification of superior allele(s)/haplotypes, which can be deployed in breeding through modern breeding approaches including marker-assisted backcrossing (MABC), haplotype-based breeding (HBB) and genomic selection (GS)
Abbreviations: HPC = high-performance computing, QC = quality check
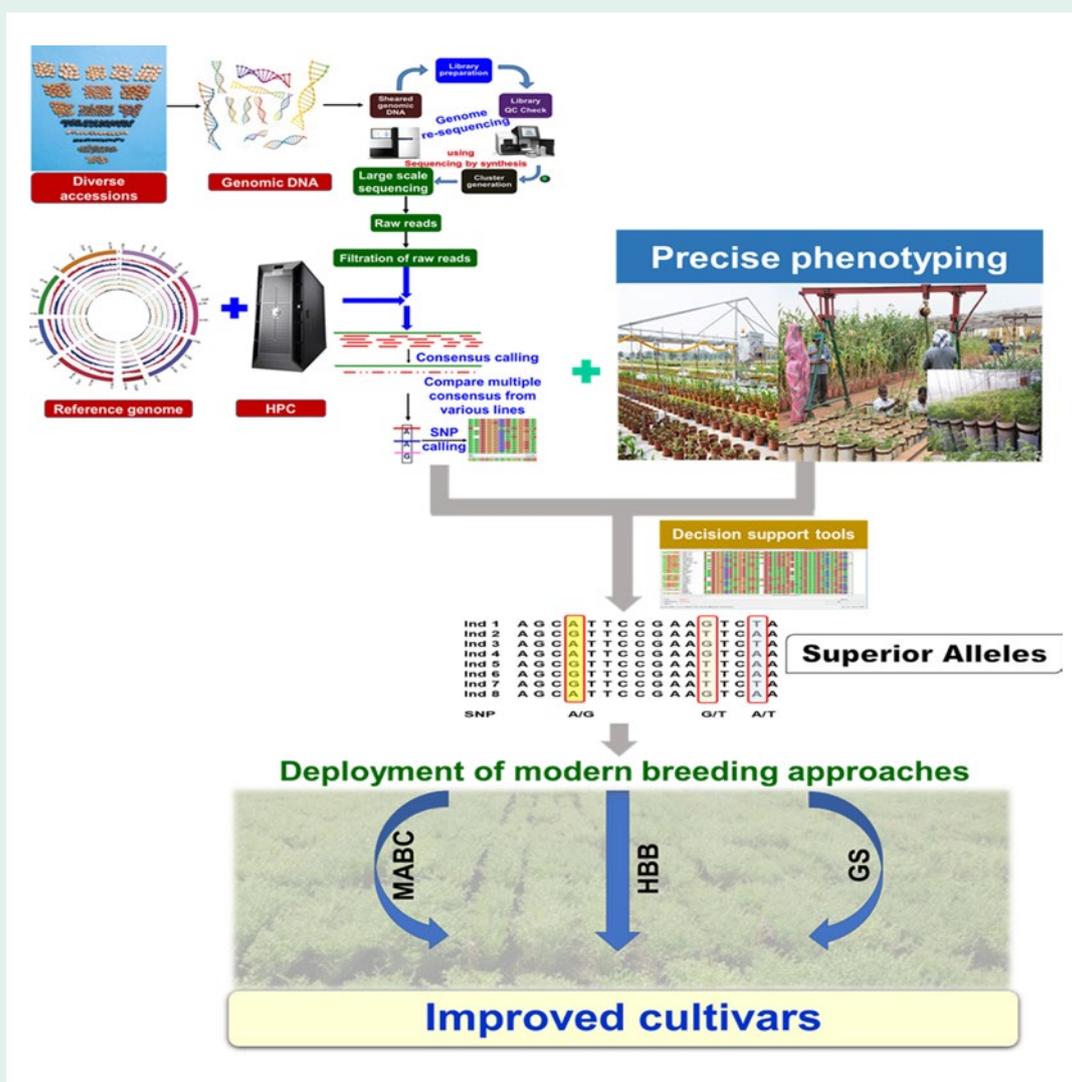
Table 2. List of chickpea molecular breeding lines released for commercial cultivation

No.	Variety	Background	Trait/ advantage	Country of release	Year of release
1.	Geletu	JG 11	Yield 15%	Ethiopia	2019
2.	Girar	JG 11	Yield 22.8%	Ethiopia	2020
3.	Pusa Chickpea 10 216	Pusa 372	Drought 11%	India	2019
4.	Pusa Chickpea 4 005 (BG 4 005)	Pusa 362	Drought 11.9%	India	2021
5.	IPC L4-14 (IPCMAS-1)	DCP 92-3	Drought 14.8%	India	2021
6.	Pusa chickpea Shyam	JG 16	Drought 16%	India	2022
7.	IPCMB 19-3	Pusa 256	Fusarium wilt 30.3%	India	2021
8.	Pusa Chickpea Manav	Pusa 391	Fusarium wilt 28%	India	2020
9.	Super Annigeri 1	Annigeri 1	Fusarium wilt 7%	India	2019

Ethiopia

Geletu, bred using GAB, was released for commercial production in the dry semi-arid to moist agroecological zones of Ethiopia by the country's National Variety Release Committee in 2019. With a grain yield of 3 822 kg/ha, it demonstrated a 15 percent yield advantage over the check variety 'Teketay' and 78 percent over the local check variety (ICRISAT, 2019). Similarly, another chickpea variety Girar (ICCMABCA-39), was released for commercial cultivation in Ethiopia by the Gondar Research Center (regional centre) of Amhara Institute of Agricultural Research in Ethiopia in 2020. With an average yield of 2 140 kg/ha, Girar has shown 22.8 percent, 41.7 percent, and 95.5 percent yield advantage over Dimtu, Natoli and local check, respectively.

A farmer holding chickpea of the Gelatu variety in her field in Ethiopia, together with Dr Asnake Fikre, Lead Legumes Researcher, Ethiopian Institute of Agricultural Research

©Asnake Fikre

India

A desi chickpea variety, Pusa Chickpea 10 216 (BGM 10 216), which was developed by ICAR-IARI using MABC to introgress the "*QTL hotspot*" for drought tolerance, was released for the Central Zone of India in 2019 (Bharadwaj *et al.*, 2022). It had an 11 percent yield advantage over the recurrent parent Pusa 372 (ICAR, 2019).

By deploying MABC, BG 4 005 was developed with a "*QTL hotspot*" for drought tolerance by ICAR-IARI in collaboration with ICRISAT. BG 4 005, having 22.4 g 100 seed weight and 131 days of maturity, reported an average of 1.6–1.7 tonnes/ha yield to report a 11.9 percent yield advantage over its recurrent parents under rainfed conditions.

A farmer holding chickpea of the Pusa Chickpea 10216 variety in his field in India

©ICAR-IARI/Chellapilla Bharadwaj

ICAR-IIPR collaborated with ICRISAT to develop IPC L4-14 using MABC to introgress drought tolerance. IPC L4-14, with 16.6 g 100 seed weight and 128–133 days maturity, reported an average yield of 1.5–1.6 tonnes/ha. In the AICRP trials, IPC L4-14 reported 14.8 percent overall weighted mean yield advantage over the recurrent parents under rainfed conditions and was released for commercial cultivation in India in 2021 (ICRISAT, 2021).

During 2022, another MABC drought tolerant introgression line, Pusa chickpea Shyam, developed by ICAR-IARI in collaboration with ICRISAT, was released for commercial cultivation in India. Based on multi-location trails under AICRP, Pusa chickpea Shyam recorded an overall weighted mean yield advantage of 16 percent over the recurrent parents.

IPCMB 19-3 with enhanced fusarium wilt resistance was developed by ICAR-IIPR in collaboration with ICRISAT using MABC. IPCMB 19-3, with 24.1 g 100 seed weight and 106 days of maturity, reported an average yield of 2.0–2.1 tonnes/ha with an overall weighted mean yield advantage of about 30.3 percent over the recurrent parent.

In 2020, the high-yielding fusarium wilt-resistant variety, Pusa Chickpea Manav (Pusa Chickpea 20211), developed by ICAR-IARI in collaboration with ICRISAT using MABC, was released for commercial cultivation in the Central Zone of India. With enhanced resistance to Fusarium wilt, its average yield of about 2,400 kg per hectare was 28 percent higher than that of its recurrent parent, Pusa 391 (ICRISAT, 2020).

The Super Annigeri 1 (MABC-WR-SA-1) variety, which was developed by the University of Agricultural Sciences Raichur in collaboration with ICRISAT by introgressing fusarium wilt resistance in Annigeri 1, demonstrated a 7 percent increase in yield over its recurrent parent and was released for commercial cultivation in the Central Zone of India in 2019 (ICAR, 2019).

Kenya

For Kenya, introgression of a "*QTL-hotspot*" into the improved varieties Chania Desi 1, Chania Desi 2 and LTD 068, Saina K-1 resulted in six promising lines, MABC 7, 9, 10, EUC-03-P6-2-2-2-8, EUC-03-22-1-2-7-13 and EUC03-P6-1-3-9-2, with improved 100 seed weight and grain yield relative to the recurrent parents (Kosgei *et al.*, 2022). Based on multilocation trials, three best genotypes EUCK-6, EUCD-P6 and EUCD-P52 have been selected for the Kenya Plant Health Inspectorate Service national performance trials (2020-2021) for possible release in 2023.

Chickpea lines developed through marker-assisted backcrossing in Kenya, with Dr Paul Kimurto, Assistant Professor, Egerton University, in the foreground

©Egerton University

The United Republic of Tanzania

The collaborative efforts of the Ethiopian Institute of Agricultural Research and the Tanzania Agricultural Research Institute have facilitated sharing of some of the MABC lines of chickpea (having the "*QTL-hotspot*" introgressed into them) with the agricultural research programme of the United Republic of Tanzania. The station trials conducted by the Ukiriguru Centre, Tanzania Agricultural Research Institute for two consecutive years (2018 and 2019) with participation from farmers in selection decisions have led to the identification of farmers-preferred lines carrying drought tolerance traits. The set of top-performing chickpea lines includes MABC 1, MABC 8, MABC 15 and MABC 21. The on-farm testing of these high-yielding and drought tolerant lines has been planned in 2023 for possible release in the United Republic of Tanzania.

Farmers participatory varietal selection trials of chickpea lines produced by marker-assisted backcrossing in the United Republic of Tanzania

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Conclusion

With changing climate scenarios, serious challenges are emerging as a threat to ongoing genetic improvement programmes. Although there is a lot of advancement in genomics that has resulted in cost reduction and accuracy, phenotyping is still a challenge. Even today, precise and high throughput phenotyping lacks several important traits that need to be optimized to improve the selection efficiency through a phenotypic-based genomics-based selection of breeding lines. The current breeding programmes for most food security crops do not seem able to generate improved crop varieties fast enough to keep pace with, or preferably be ahead of, the persistently evolving constraints to crop production systems. Towards this end, the rates of genetic gain in breeding for improved productivity must be maintained or even increased.

Modern approaches such as genomic prediction-based selection, early generation selection, haplotype-based breeding, genome editing and rapid generation advancement have shown their potential to accelerate the rate of genetic gain and improve crop improvement efforts using less time and fewer resources (Varshney *et al.*, 2021a). Translating the crop productivity gains from experimental settings to real world farming conditions seeks a substantial improvement in farmers' access to these innovative technologies and their adoption.

In this context, Pusa 10 216 and Pusa Chickpea Manav varieties are likely to cover 31 218 ha and 43 032 ha respectively in India over the next three years (by 2026–27). These estimates are derived based on the current seed rate of chickpea, and standard conversion rates from breeder-to-foundation and foundation-to-certified seed in India. The breeder seed indents (i.e. the written requisitions submitted by seed producers for breeder seed of released cultivars), received through the Department of Agriculture and Farmers' Welfare, India over the last three years for Pusa 10 216 and Pusa Chickpea Manav are 11.1 tonnes and 15.3 tonnes respectively. This also reflects the growing demands for these improved varieties among Indian farmers.

The improved varieties of chickpea developed through MABC will contribute to enhanced income of smallholder chickpea farmers in India. For instance, we anticipate that, in India, Pusa 10216 and Pusa Chickpea Manav in the next three years will contribute USD 19.1 million and USD 61.1 million respectively in terms of their export value in comparison to standard chickpea varieties. This was calculated using the expected area covered by these two varieties over the next three years, the gains (kg) per hectare based on 70 percent of their potential yields (Pusa 10 216: 2 575 kg/ha, Pusa Chickpea Manav: 3 915 kg/ha), figures from FAO (2021) for the national crop productivity in India for chickpea (1 088 kg/ha) and export prices of USD 0.86 per kg (Selina Wamucii, 2022).

As is evident from temperate cereal crops, a robust seed system delivering improved cultivars steadily in farmers' field to replace the old cultivars offers the most obvious means to adapt agriculture to climate change (Atlin, Cairns and Das, 2017). We advocate strengthening or establishing (if they don't exist) robust seed delivery systems, use of informed production and harvesting technologies that help to realize higher crop yields, generate more income and deliver better products (Varshney *et al.*, 2021b).

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3.5 Developing and deploying climate-smart maize and pod borer resistant cowpea for smallholder farmers in Africa

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Abstract

Frequent droughts and outbreaks of insect pests are significant constraints to the gainful production of the major staple crops, maize and cowpea in sub-Saharan Africa. The routine access to agricultural biotechnologies, which could address these constraints, is limited in the region. In this case study, we describe the brokered royalty-free access to proprietary biotechnologies and their subsequent use in the genetic improvement of these two food security crops. Modern plant breeding techniques, including doubled haploidy and molecular marker-assisted breeding, were used to develop over 120 conventionally bred climate-smart DroughtTEGO® maize hybrids, which were released mostly in East and Southern Africa. Transgenic TELA® maize hybrids that were resistant to fall armyworm, stem borers and drought were also developed in Ethiopia, Kenya, Mozambique, Nigeria and South Africa. Additionally, transgenic cowpea varieties with resistance to pod borer insects were developed; the SAMPEA 20-T variety was released in Nigeria. All these improved varieties had significantly higher yields than the commercial check varieties, especially under the target stress conditions. The adoption of the insect-resistant varieties led to significant reductions in the use of expensive and harmful insecticides. Overall, there were significant improvements to the livelihoods of farmers, typically smallholders, who adopted these improved crop varieties. Partnerships, especially between the public and private sectors, sustained funding and political goodwill were critical to the successful development and deployment of these solutions.

Introduction

Maize (*Zea mays* L.) is an important food security crop for over 300 million people in sub-Saharan Africa (SSA). The frequent incidents of drought and damage by insect pests, especially stem borers (*Chilo partellus*, *Busseola fusca* and *Sesamia calamistis*) and, most recently, fall armyworm (FAW, *Spodoptera frugiperda*), which are associated with climate change, pose significant threats to the production of the crop. On average, drought causes a yield loss of about 17 percent for maize in the region. Compounding this, stem borers infestation reduces maize production by an average of 13 percent or 400 000 tonnes of maize per year, for instance in Kenya. This is equivalent to the country's annual import of the crop, valued at over USD 90 million (De Groote, 2002). First observed in the continent in 2016, FAW, a devastating and transboundary pest, if not controlled adequately, could cause yield losses of up to 20 million tonnes of maize annually, which is enough to feed 100 million people (CABI, 2017).

Cowpea (*Vigna unguiculata* [L] Walp.), commonly known as black-eyed pea, is also a staple crop for over 200 million households in SSA (Kamara *et al.*, 2016). The crop's protein-rich grains, green leaves and immature pods are all eaten, with the grains being used as important weaning food for babies (Bassey *et al.*, 2013). Pod borer (*Maruca vitrata* F) is one of the major insect pests of the crop, with its infestation causing significant yield losses of 20 to 80 percent (Addae *et al.*, 2020). With no known varieties that are resistant to *M. vitrata,* smallholder farmers, the main producers of the crop, spray their farms several times each season with expensive synthetic insecticides (Murdock *et al.*, 2008) which, in addition to being detrimental to the environment and human health, are usually ineffective against the insect's larvae (Ba *et al.*, 2019).

In 2004, the African Agricultural Technology Foundation (AATF), with headquarters in Nairobi, Kenya, brokered access to a royalty-free license from Bayer (then Monsanto Company) to use its proprietary technologies for the genetic improvement of these food security crops in SSA. These were the Cry series of genes, Cry1Ab and Cry1A.105/Cry2Ab2, isolated from the bacterium *Bacillus thuringiensis* (Bt); and the CspB gene, a cold shock protein isolated from the bacterium *Bacillus subtilis*. The innovative use of genetic engineering or genetic modification, based on these genes, and molecular breeding approaches to develop varieties of these two food security crops, which are resistant to these production constraints, is described in this case study.

Climate-smart maize: Product development, commercialization and impacts

Product development

The aim of the Water Efficient Maize for Africa (WEMA) Programme, which commenced in 2008 and was renamed the TELA Maize Programme in 2018, was to develop and deploy new climate-smart maize hybrids for smallholder farmers that would be both drought-tolerant and insect-resistant through an innovative public-private partnership (PPP) (Oikeh *et al.*, 2015). The partners and their contributions are summarized in Table 1. Anchored by the guiding principle that the products of biotechnology are safe and could contribute to enhanced food security and nutrition, the partnership benefits from long-term investments by the Bill and Melinda Gates Foundation (BMGF), the Howard G. Buffett Foundation (HGBF) and the United States International Development Agency (USAID).

The climate-smart varieties were developed using both non-transgenic breeding and transgenic techniques. For the former, doubled haploidy, i.e., the chromosome doubling of haploid cells was used to develop homozygous breeding lines in one generation (compared with up to six generations through selfing). The molecular marker-assisted breeding (MAB) techniques, marker-assisted recurrent selection (MARS) and genome-wide selection (GWS), were used to further increase the efficacy and speed for breeding the improved varieties (Spindel *et al.*, 2015). Definitions of these and other main biotechnologies described in this case study are provided in Box 1.

For the genetic engineering techniques, the *CspB* gene, which confers drought tolerance, was used to transform maize, with the resulting transformation event and trade name being MON87460 and DroughtGard® respectively. Similarly, the resulting transformation events using the insect resistance genes *Cry1Ab* and *Cry1A.105/Cry2Ab2* were Bt MON810 and MON89034 respectively. These conferred resistance to FAW and stem borer in already adapted conventionally bred drought-

Box 1. Definitions of technical plant breeding terms

Doubled haploids (DH) technology: Doubled haploid (DH) technology is used in plant breeding to rapidly develop true breeding plant lines by chromosome doubling of haploid cells. Haploid cells contain a single set of chromosomes. Haploid cells are produced from pollen (male) or egg (female) gametes or plant sex cells. Doubled haploids (DH) are produced when the haploid cells undergo either induced or spontaneous chromosome doubling. The doubled haploid cell can then be grown into a doubled haploid plant. Conventional inbreeding procedures take six generations to achieve approximately complete homozygosity (the presence of two identical alleles at a particular gene locus) or true breeding status, whereas doubled haploidy achieves it in one generation.

Molecular markers-assisted breeding (MAB): Classical plant breeding is the intentional interbreeding and selection of plant varieties with the goal of producing new varieties with improved properties. Marker-assisted breeding (MAB), more precisely molecular markers-assisted breeding, combines classical plant breeding with the tools and discoveries of molecular biology and genetics, most specifically the use of molecular markers. Molecular markers are variants in the DNA sequence which acts as an identifier or tag of a particular aspect of phenotype and/or genotype and its inheritance can easily be followed from generation to generation.

Marker-assisted recurrent selection (MARS): Marker-assisted recurrent selection (MARS) is a breeding method used to accumulate favourable alleles within a single population. In MARS, marker-assisted selection is used for three or more cycles in a plant population to improve traits governed by more than two genes as a means of increasing the frequency of the desirable genes for various economic characters. It helps in maintaining the high genetic variability in the heterozygous population and increases the efficiency and effectiveness of the population improvement by avoiding the influences of environmental effects.

Genome-wide selection (GWS): Genome is the complete set of DNA (genetic material) in an organism. The genome contains all the information needed for the organism to develop and grow. Genomic selection involves making use of the estimated association between many markers and the phenotype to estimate the breeding value of the plant without phenotype. A genome-wide association study (GWAS) is a research approach used to identify genomic variants that are statistically associated with a particular trait. GWAS explores the complete genome, in contrast to other approaches that exactly investigate a minor amount of pre-specified chromosomal areas. Genomic selection is a form of marker-assisted selection in which genetic markers covering the whole genome are used so that all quantitative trait loci (QTL) are in linkage disequilibrium with at least one marker. Genome-wide selection, therefore, is based on all the good genetic make-up of a plant for a particular trait.

Genetic engineering: Genetic engineering (also called genetic modification) is a process that uses laboratory-based technologies to alter the DNA makeup of an organism. This may involve changing a single base pair (A-T or C-G), deleting a region of DNA, or adding a new segment of DNA. For example, genetic engineering may involve adding a gene from one species to an organism from a different species to produce a desired trait. Used in research and industry, genetic engineering has been applied to the production of genetically modified plants.

Source: Dr Stephen Mugo, Independent Consultant Breeder, TELA Maize Programme Advisor, Personal Communication, 2022.

Table 1. Partners and their contributions to the WEMA/TELA Maize Programme

Organization	Contribution
AATF	- Overall coordination of the PPP - Brokerage of royalty-free licensing - Product deployment and stewardship - Communication, outreach and advocacy - In-country regulatory approvals and compliance - Monitoring and evaluation
International Maize and Wheat Improvement Center (CIMMYT)	- Provision of crop germplasm adapted to SSA - Conventional breeding, including the use of marker-aided selection and doubled haploidy, for tolerance to biotic and abiotic stresses - Field testing, including for regional trials
Bayer Crop Science	- Provision of the genes Cry1Ab and Cry1A.105/Cry2Ab2 for insect resistance and CspB for drought tolerance, royalty free - Provision of crop germplasm adapted to different regions of the world - Conventional breeding, including the use of marker-aided selection and doubled haploidy, and genetic engineering for tolerance to biotic and abiotic stresses - Biotechnology testing and stewardship - Seed production, deployment and licensing - Regulatory data packages
National agricultural research system (NARS) of: - Ethiopia - Kenya - Mozambique - Nigeria - South Africa - Uganda - United Republic of Tanzania	- Provision of locally adapted germplasm - Conventional breeding for tolerance to biotic and abiotic stresses - Field testing, including for regional trials - Knowledge of farmers' product needs

tolerant varieties. The inclusion of the drought tolerance gene further enhanced yield under drought stress conditions by at least 10 percent (Castiglioni *et al.*, 2008). Bayer conducted the genetic transformation in its facilities in Mexico.

Subsequently, Bayer South Africa and the Agricultural Research Council (ARC), the South African NARS, crossed the drought-tolerant and insect-resistant transgenic variants with elite maize parental lines. The promising maize varieties with the incorporated transformation events were further trialled in the seven programme countries (Table 1) in collaboration with the respective NARS and other programme partners.

The 124 hybrid maize varieties developed through non-transgenic breeding and released to farmers were trademarked DroughtTEGO® (TEGO means shield in Latin). The transgenic varieties, developed with Bt only or Bt stacked with drought tolerance traits, were called TELA® (from the Latin word *Tutela*, meaning protection). A mean genetic gain of 70.5 kg per hectare per year, which was obtained for DroughtTEGO® hybrids from using GWS, was two to four times higher than those ever reported from conventional or non-transgenic breeding in SSA (Beyene *et al.*, 2015).

A farmer in Kitali, Kenya, in October 2018 identifies the non-transgenic DroughtTEGO® WE6105 hybrid as the best performing DroughtTEGO® hybrid in her demonstration plots for the Highlands agroecology in western Kenya

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Relative to isogenic conventionally bred hybrids, under combined FAW and stem borer infestation, on average, TELA® hybrids with Bt MON810 had 43 percent yield advantage while, under moderate drought-stress, TELA® with MON87460 trait gave about 17 percent yield advantage. But under optimum moisture conditions with chemical control of pests, TELA® hybrids produced similar yield as conventional isogenic hybrids, confirming that there were no unintended changes due to genetic transformation. These promising results strengthened the applications for the environmental release of the transgenic insect-resistant and drought-tolerant events in Ethiopia, Kenya, Mozambique, Nigeria and South Africa.

In Makhatini, South Africa, Dr Kingstone Mashingaidze, a maize breeder from the Agricultural Research Council, showcases in October 2019 the performance of the TELA® WE6208B Bt hybrid (right) vs. the non-transgenic, drought tolerant, DroughtTEGO® WE3128 hybrid (left) under combined moderate drought and severe fall armyworm and stem borer infestation. The WE6208B hybrid had a six-fold higher yield than the WE3128 hybrid

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Product deployment and commercialization

Since 2016, five TELA® maize varieties have been commercialized in South Africa, where they have been adopted by smallholder farmers. These varieties are contributing significantly to the management of FAW in the country. At the time of preparing this article (November 2022), the approval processes for the commercialization of three TELA® maize varieties were ongoing in Kenya, while promising results have been obtained for 12 varieties, which are undergoing variety certification trials in Nigeria.

To get the improved maize varieties to farmers, AATF followed a business model which was used first for the commercialization of DroughtTEGO® maize varieties in Kenya in 2013. The model involves, among other components: the establishment of demonstration plots for farmer participatory variety selection; issuing humanitarian-use licenses for the varieties to small- and medium-enterprise (SME) seed companies; and establishing linkages with early generation seed suppliers such as two AATF's subsidiaries, QulaiBasic and EcoBasic seed companies for the production of foundation seeds. The business model also includes developing an account management system, involving technical backstopping on seed production and business support for licensed SME seed companies. The provision of stewardship support, involving the preservation of product integrity by adopting standard operating procedures, quality control and assurance, and insect resistance management to delay the development of resistance by the target pests, is also an integral part of the business model.

Within five growing seasons, in less than three years, over 4860 demonstration plots were established across 17 counties in Kenya. The yields of DroughtTEGO® varieties, which ranged from 5.5 to 6.3 tonnes per hectare, were 33 to 54 percent higher than those of the commercial check varieties (Obunyali *et al.*, 2019). The experience and lessons learned were used in the release of the varieties in other non-programme countries, Benin, Cameroon, Ghana, Rwanda, Zambia and Zimbabwe, in particular through the Technologies for African Agricultural Transformation (TAAT) programme funded for three years by the African Development Bank.

Impacts of DroughtTEGO® and TELA® Maize

The issuance of humanitarian-use licenses to 38 SME seed companies to commercialize DroughtTEGO® and TELA® varieties was one of the key success factors for impact. Through the account management system that was put in place, the annual volumes of certified seeds that were produced by licensed SME seed companies were tracked. It was estimated that between 2013 and 2020, over one million hectares were sown with over 29 000 tons of certified seeds of these improved maize varieties in 13 countries, Benin, Cameroon, Ethiopia, Ghana, Kenya, Mozambique, Nigeria, Rwanda, South Africa, the United Republic of Tanzania, Uganda, Zambia and Zimbabwe (Table 2).

Table 2. The volume of seeds (tonnes) of the DroughtTEGO® (non-transgenic drought tolerant) and TELA® (transgenic drought tolerant and/or insect resistant) maize varieties produced by SME seed companies on the strength of humanitarian-use licenses issued by AATF in 13 countries from 2013 to 2020 through the WEMA/TELA or the TAAT programme

Year	Volume of seeds produced by programme/product			
	WEMA/ DroughtTEGO®	TELA/TELA®	TAAT/ DroughtTEGO®	TOTAL
2013	75	-	-	75
2014	217	-	-	217
2015	1 986	-	-	1 986
2016	1 998	5	-	2 003
2017	947	56	-	1 003
2018	533	97	930	1 560
2019	685	28	21 031	21 744
2020	591	5	22	618
TOTAL	7 032	191	21 983	29 206

Source: AATF Deployment and Commercialization Database, 2021.

In 2021, AATF commissioned an external impact evaluation of the WEMA programme in Kenya, the United Republic of Tanzania and Uganda. It was shown that the productivity of maize and the consequent income generation for farmers increased significantly with the adoption of these new varieties. In Kenya and Uganda, for instance, the cultivation of DroughtTEGO® led to an average yield increase of 248 percent while income generation was enhanced by 84 percent (AATF, 2021). The hybrids were recently described as 'magic seeds' (BMGF, 2022) because they were already enhancing the livelihoods of farmers in both countries. These significant improvements were not observed in these two indicators in the United Republic of Tanzania, possibly because deployment started very late in this country, a couple of years before the closure of the WEMA programme in 2018.

Pod borer resistant cowpea: Product development, commercialization and impacts

Product development

Enabled by funding from the USAID and the Rockefeller Foundation, a PPP was formed to facilitate the use of the Cry1Ab gene to develop pod borer resistant (PBR) cowpea varieties for deployment to smallholder farmers (Table 3).

The Cry1Ab gene was used to transform the cowpea variety IT86D-1010 in CSIRO where the efficacy against the *Maruca* pest was demonstrated (Higgins *et al.*, 2012). Subsequently, several variants with the incorporated Cry1Ab-expressing events were tested in a confined environment under severe *Maruca* artificial infestation in Nigeria, Burkina Faso and Ghana. Scientists at the NARS in Nigeria, Burkina Faso and Ghana used a variant known as Event 709A, identified as the most promising, as the parental line to introgress the insect resistance trait into farmer-preferred varieties.

The resulting transgenic PBR cowpea varieties were evaluated in confined field trials from 2011 to 2016 in the three countries (Addae *et al.*, 2020). The confined field trials results showed consistently that there was nearly a complete protection from *Maruca* for the pods and seeds of transgenic cowpea varieties. In most cases. there were also significant increases in grain yield over non-Bt control lines. Also, with the transgenic varieties, the frequency of the spraying of insecticides to control the pests was reduced from six to ten times per season to just two, representing a saving of four litres of insecticide per hectare or USD 12.5 per hectare.

In Nigeria, after the approval by the National Biosafety Management Agency for commercial release in January 2019, followed by successful variety certification trials across cowpea growing agroecologies, the first PBR cowpea variety, known as SAMPEA 20-T, was registered and released for cultivation in December 2019 (Addae *et al.*, 2020). Transgenic PBR cowpea was also approved for environmental release towards commercialization in Ghana in June 2022 by the National Biosafety Authority following which national multi-locational performance trials for the registration and release of the promising varieties were commenced. In Burkina Faso, it was envisaged that the regulatory dossier for its environmental release would be submitted to the biosafety regulatory authority for review and decision making in December 2022.

**Table 3. PBR cowpea project partners and roles in product development
and deployment**

Organization	Roles
AATF	AATF coordinated and managed the PPP; brokered royalty-free access to the proprietary Bt technology and the ensuing varieties; provided expertise and support in product development, regulatory compliance, business development, seed delivery and stewardship, communication and outreach.
Bayer Crop Science (then Monsanto Company)	Donated the proprietary biotechnology, i.e. the Bt gene construct, used for cowpea transformation on a royalty-free basis
Purdue University, through the Network for Genetic Improvement of Cowpea for Africa (NGICA)	Provided technical backstopping to the project at product development phase
Commonwealth Scientific and Industrial Research Organization (CSIRO), Australia	Responsible for the genetic transformation of cowpea and the generation of the variants for field testing
Donald Danforth Plant Science Center	Supported the development of the regulatory dossier for commercial approval of PBR cowpea in project countries
Institute of Agricultural Research, Ahmadu Bello University, Samaru, Nigeria	Conducted field testing and variety development in Nigeria
Council for Scientific and Industrial Research – Savana Research Institute, Ghana	Conducted field testing and variety development in Ghana
Institute de l'Environnement et de la Recherche Agricole, Burkina Faso	Conducted field testing and variety development in Burkina Faso
International Food Policy Research Institute, Program for Biosafety Systems	Supported regulatory compliance training in project countries

Product deployment and commercialization

When the SAMPEA 20-T cowpea variety was launched in Nigeria in 2021 by AATF, in collaboration with the Institute of Agricultural Research, Samaru, Nigeria and other Nigerian government agencies such as the National Agricultural Seed Council, a similar business model as described for maize earlier was also followed. In the first year of commercialization, smallholder farmers planted 7 tonnes of the seeds. The seed delivery system involved the issuance of humanitarian-use licenses for the SAMPEA 20-T variety to three SME seed companies and the establishment of product stewardship, and a seed systems advisory package to support the seed and crop production process. An insect resistance management plan for Bt cowpea which required non-Bt cowpea refuge fields to be planted in proximity to the Bt cowpea fields was recommended. The aim was to provide sources of susceptible insects from the non-Bt refuge fields, which can breed with insects that may survive exposure to the Bt protein, thus delaying the development of insect pest resistance to the Bt protein. A community or village refuge insect resistance management approach was implemented, whereby non-Bt cowpeas and landraces planted by neighbouring farmers provided sources of

Mr Bernard Ehirim, African Agricultural Technology Foundation, inspects the performance of pod borer resistant SAMPEA 20-T cowpea seed production in Philip Ibrahim's farm in Mokwa, Niger State, Nigeria in November 2022. The field was completely free of pod borers and good harvest was assured

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insect pests (*M. vitrata),* which were susceptible to Cry1Ab protein, in order to prevent the evolution of resistant biotypes.

More recently, additional funding was provided by the BMGF for the out-scaling of the commercialized transgenic variety in Nigeria in response to the bourgeoning demand by smallholder farmers.

Potential impacts of PBR cowpea

Ex ante impact assessment using an economic surplus partial equilibrium model estimated a total net present value (NPV) of adopting PBR cowpea varieties that ranges from USD 5.6 million to USD 125.4 million over a 30-year period in Ghana (Dzanku *et al.*, 2018). The net present value benefits of adopting PBR cowpea for producers and consumers were estimated at about USD 350 million, with 70 percent accrued by producers over a period of 25 to 35 years in Nigeria (Dayo *et al.*, 2019).

Major challenges in developing climate-smart maize and PBR cowpea

The key challenges encountered in developing these new varieties included the replication of managed drought stress assays in confined field trials across countries in formats that were suitable for data transferability. Secondly, in some countries, there were prolonged delays in getting government approvals for environmental release of the transgenic varieties, which was needed for variety certification trials and ultimately obtaining approval to commercialize them. Finally, there has been increased anti-biotech activism in Africa aimed at preventing African farmers from adopting transgenic crop varieties.

Lessons learned in developing climate-smart maize and PBR cowpea

The use of a PPP model for product development and deployment enabled access to critically important proprietary biotechnological tools and expertise as well as diverse elite germplasm from public and private partners, which otherwise would not have been available to a single partner organization.

The holding of licensee SME seed companies accountable for attaining high seed quality standards through humanitarian-use licenses and account management systems and detailed stewardship measures, with compliance audits among farmers, were effective ways to enhance the efficiency of product deployment and ensure its sustainability.

The securing of the approval for general releases of transgenic crops in Africa is difficult and complicated, but not impossible if the requisite legal and regulatory framework is accompanied by consistent and supportive political goodwill.

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3.6 Use of tissue culture for the propagation of two economically important native plants in a forest community home gardens model in Nigeria

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Abstract

Native plants have the potential to scale up food security/nutrition and biodiversity conservation due to their potential contribution to preventing malnutrition, obesity and diet-related disorders as well as hidden hunger. Sadly, most of them are found in restricted local areas and may become extinct before they are even discovered, with their enormous associated traditional knowledge disappearing with them. In response to this, we conducted experiments on models to provide alternative sources of propagation of these plants to help boost domestic and economic livelihoods while reducing pressure on the forest around the community. Tissue culture, a biotechnology tool, was chosen as an alternative to the propagation of these plants. The attraction of tissue culture as an alternative to other propagation methods lies in its ability to mass multiply plant materials very rapidly. Tissue culture shortens the plant's development time while producing plantlets that would have long overcome the effects of pests and diseases at the seedling stage. Tissue culture of two economically important native plants, *Gnetum africanum* (Okazi) and *Gongronema latifolium* (Utazi), was applied and they were mass multiplied for use to develop home gardens for the Umuanunu forest community in Owerri West, Imo State, Nigeria. The project successfully increased the availability of these plants in the community, reduced their exploitation in the forest and increased people's access to nutrient-rich food. They also have maximized the potential of these plants in terms of market and sales which has been sustained by a model approach that combined their traditional knowledge with science and technology.

Introduction

Africa's forests have special meanings to native cultures that have inhabited them for millennia, where customary law gives locals the right to use trees for fuel and construction, and to collect forest products for food and medicine. Consequentially, many plant species are threatened and listed as critically endangered (Eneobong, 1997). Sadly, the pressure on forest flora in this region continues to increase and biodiversity is being lost at alarming rates (Oko, Odey and Anake, 2022).

Wild edible plants are part of non-wood forest products and they constitute a major natural resource for the rural poor. They also play important roles in the economy of the rural communities and their daily lives. These plants are fully recognized at the local level and are harnessed to support household food security and provide income as well as pharmaceuticals and other therapeutic

materials (Burkill, 1994). However, it has been pointed out that uncontrolled market explosion leads to over-harvesting of these resources hence strategies that encourage market expansion by boosting the resource base, such as providing a sustainable system for the cultivation and domestication of these plants, should be in place to enhance their availability and marketability.

Gnetum africanum (Family: Gnetaceae) is a traditionally wild perennial vine, known by a number of local and trade names; Afang in Efik, Okazi among the Ibos, Eru in Western Cameroon and Koko in French (Bahuchet, 1990). Leaves of *Gnetum africanum* have high nutritional and medicinal values (Ali, Assanta and Robert, 2011). A highly valued household vegetable, collected more from the wild (rather than farmed) across the tropics, *Gnetum africanum* is very rich in proteins and minerals. The leaves are highly nutritious containing eight essential amino acids (Mialoundama, 1993).

Gongronema latifolium (Family: Apocynaceae) is a plant that has a wide range of nutritional and ethnomedical uses in different tropical African communities. It is commonly called 'Utazi' in the southeastern parts of Nigeria and 'arokeke' in southwestern Nigeria (Ugochukwu *et al.,* 2003). The leaves are rich in phytochemicals, fats, proteins, vitamins, minerals and essential amino acids. The plant is commonly used in soup as a vegetable, or dried and applied as a powdery spice. It is also consumed fresh and can be used in salad preparations.

It is equally important to note that *Gnetum africanum* and *Gongronema latifolium* plants are forest climbers and generate household incomes for rural women and unemployed youths in the Umuanunu forest community in Owerri West, Imo State and are both considered to be a ceremonial delicacy in the community. Sadly, over-dependence on these plants for purposes of food and household income has rendered them vulnerable to exploitation as they are harvested in large volumes to meet local demands. Since most of these plants remain wild, they suffer a high extinction risk necessitating alternative means of rapid mass propagation while promoting their conservation in the wild (Uyoh, Nkang and Eneobong, 2003).

Biotechnology as a solution

Biotechnology is an important tool for conservation of plant bioresources. As many of these wild plants are hard to propagate due to their production of seeds with little or defective endosperms, the method of plant tissue culture (micropropagation) provides an option for their mass clonal propagation (Uyoh, Nkang and Eneobong, 2003). This case study discusses our project carried out between 2008 and 2010 using the micropropagation technique for the propagation and conservation of these two economically important endangered local plant species in a forest community home gardens model in Nigeria.

Tissue culture (micropropagation) optimization

According to Kumar (2018), media development and optimization in tissue culture is a difficult process, which encompasses satisfactory alteration of all components to their best concentrations to increase viability as well as promote vigorous growth in the culture. Several essential components and supplements in media formulation (such as growth factors, vitamins, hormones, trace elements and other micronutrients) are individually optimized based on different growth requirements of specific cell types.

Various optimization protocols for micropropagation were carried out at the Biodiversity and Climate Research Laboratory, Biotechnology Advanced Research Centre at the Sheda Science and Technology Complex between 2006 and 2008 for the two species. The process of micropropagation can be summarized in the following stages as shown in Figure 1.

Explant preparation

The process of micropropagation starts with the selection of plant tissues commonly called "explants" from a healthy, vigorous mother plant. For our experiments we collected the leaves and apical meristems of the plant as explants to be used for the cultures. Explants of the plants were taken from forest plants of the Umuanunu forest community in Owerri West, Imo State, put in sterile distilled water and then transported to the Sheda Science and Technology Complex laboratory for further studies.

Sterilization

In this stage, explants were surface sterilized at different concentrations of ethanol and sodium hypochlorite and the best concentration included the use of the washing detergent Tween 20 used for surface sterilization of the explants. Surface sterilization of explants in chemical solutions is a vital step to eliminating contaminants with insignificant damage to plant cells.

Shoot and multiplication

The aim of this phase is to increase the number of propagules. Explants inoculated on Murashige and Skoog (MS) medium supplemented with 2.0 percent sucrose and 2.0 mg/l of N6-benzylaminopurine (BAP) and 0.5 mg/l of indole acetic acid (IAA) showed the best response for the *Gnetum africanum* shoots while the highest shoot length in *Gongronema latifolium* was observed on MS medium containing 1.0 mg/l BAP and 0.25 mg/l IAA after 15 days of inoculation.

Rooting

The rooting stage may occur simultaneously in the same culture media used for the multiplication of the explants. However, as in the case of our experiments, it is necessary to transfer shoots to another media. Optimal rooting was observed on MS medium containing 0.5 mg/l α-naphthalene acetic acid (NAA) with 2 percent sucrose within two weeks of culture transfer.

Acclimatization

At this stage, the *in vitro* plants are weaned and hardened. Hardening is done slowly from high to low humidity and from low light to high light intensity. The plants are then moved to compost and gradually hardened under the greenhouse mimicking the forest for optimal plant survival. They were then transported for use by the Umuanunu forest community.

Figure 1. The different stages of micropropagation

Explant preparation → Sterilization → Shoot and Multiplication → Rooting → Acclimatization
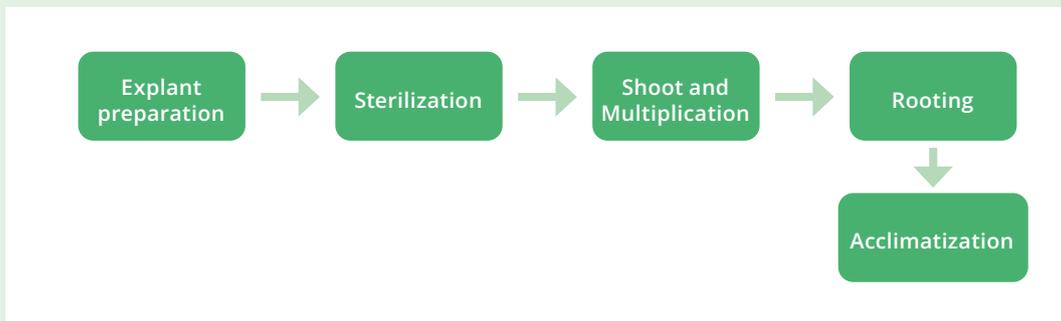
Plantlets at the rooting stage in the tissue culture laboratory of the Sheda Science and Technology Complex, Abuja

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**Plantlets of *Gnetum africanum* that were brought to the Umuanunu
forest community**

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Using tissue culture plantlets for the establishment of home gardens (the Umuanunu-Obinze project)

Generally, home gardens have been recognized as a key source contributing to food and nutritional security and livelihoods (Galluzzi, Eyzaguirre and Negri, 2010). For centuries, they have been used as integral components of family farming and local food systems (Gautam, Sthapit and Shrestha, 2006). There is increasing interest in home gardens as biodiversity hot spots as they harbour crops and their varieties that were once more widespread in the larger agroecosystem or that developed over generations in the gardens themselves, maintaining crop evolution. Their multistorey floristic composition and the multispecies structure enable niche diversity and, thus, contribute to crop evolution as well as the food production capacity at large. Furthermore, Gautam, Sthapit and Shrestha (2006) describe home gardens as buffer zones around protected areas contributing to biodiversity conservation in forest communities.

The Umuanunu-Obinze project, funded by the Global Environment Facility (GEF) Small Grant Programme (SGP) Nigeria between 2009 and 2010 (GEF-SGP, 2009), was based on studies of the over-exploitation of wild plants especially *Gnetum africanum* and *Gongronema latifolium* for their domestic and economic use. Since the majority of village people depend on these plants as their only source of livelihood, our studies had shown the alarming effects of human activities on the forest due to the collection of these plants. Most surprising is that the majority of the people in villages had complained about not having access to these plants in the forest again. Some villagers even admitted to not being aware of the detrimental effects of their collecting activities on the forest biodiversity.

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Plantlets of *Gongronema latifolium* that were brought to the Umuanunu forest community

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In response to this, experiments were conducted on models to provide alternative sources of propagation of these plants to help boost domestic and economic livelihood while reducing pressure on the forest around the community. Tissue culture was chosen as an alternative approach to propagate the two forest plant species.

The Umuanunu-Obinze project activities

A community advisory/implementation committee was set up to oversee the activities of the project. With the assistance of the project facilitators, interviews and interactions to collect information on the community's perception of conservation and adaptation to new technologies in conservation and use were carried out. A "train the trainer" workshop was organized to teach potential farmers about the handling of the new plants, including best agronomic practices for plant growth. Since both plants are forest plants, the participating farmers were taught how to mimic forest like conditions for their plants. This included providing shade for the plants to reduce the intensity of the sun and watering the plants to mimic the coolness of the forest in the home gardens.

Community members provided their own land for the proposed home gardens at their respective family units. The home gardens varied in size and averaged about 20 m x 20 m. A total of 100 community members representing 50 families took part in the project, growing only the two forest plants in the home gardens. The community has a long tradition of using home gardens, especially for tomatoes and peppers.

About 5 000 plantlets produced using tissue culture were distributed to the potential community farmers for the establishment of farms. Gender was taken into consideration in selecting the

participatory farms for the project, making sure there was more women and youth participation in the activities of the project.

Public awareness events were organized to show the acceptability of the plantlets as being safe as well as creating a market for the plants. These were organized because of some unexpected opposition from some farmers during the course of the project.

A questionnaire to gather information on how the project has affected the livelihood of the farmers and the community in general, as well as a group discussion on awareness of biodiversity loss issues within the community and to promote the use of safe technologies were also organized at the beginning and the end of the project.

Impact of the project

a. Plants' ecosystem restoration and sustainable management

The project restored plant biodiversity of *Gnetum africanum* and *Gongronema latifolium* around the Umuanunu-Obinze forest community in Owerri West, Imo State, Nigeria, where they have been depleted over time due to overexploitation, as well as contributing to preserving their traditional varieties and wild gene pools for the people. In providing an alternative source of propagation for these plants, their diversity was increased around the forest community while reducing pressure on the forest for these species. For example, at the end of the project, 20 new climbers of *Gnetum africanum* and 40 to 50 new *Gongronema latifolium* climbers of species were found in a 10 km^2 radius in the forest compared to five and ten climbers at the start of the project.

The Umuanunu-Obinze project was funded by the GEF SGP between 2009 and 2010. However, up to today, the Obinze community remains a hub for commercial production of these two forest plants via home gardens. Recent discussions by the author (in October 2022) with the head of the community, Igwe Ejimogu, indicate that more family units (about 200) have started home gardens and that sale of vegetables from both forest plant species have gone beyond their local communities to towns such as Owerri, the state capital; Onitsha, the commercial city in Anambra State; as well as Port-Harcourt, the Rivers State capital. It is worth noting from these discussions that the information of increased regeneration of these two species in the forest comes as pressure to collect them has been drastically reduced by people harvesting the species from their home gardens. It is reported that during the COVID-19 pandemic the farming of these two plants became even more popular among people in the community.

b. Gender equality

With limited access to land, women are still custodians of biodiversity with knowledge to bolster food security. Sixty-five percent of the participating community members were women, and 53 percent of the 40–65 age group and 47 percent of the 18–39 age group were women. Women's participation was supported by their husbands. This meant that the women took decisions on dietary requirements for their families and had strong participatory roles in management and benefits sharing.

Women as custodians of biodiversity. On the left, the woman is holding
Gnetum africanum

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c. Resilience and adaptability

The Umuanunu-Obinze project approach towards resilience was unique in the sense that it was able to connect communities to local plant conservation. Promoting the conservation of these plant species offered opportunities for responsible forest management and business development brought about by various training processes at the community level. Thus, armed with better knowledge, community members in their various household units were able to achieve some level of market specifications/requirements as well as access new markets i.e. well-packaged vegetables of these two species were sold in supermarkets in cities around the state capital Owerri and towards Onitsha in Anambra State. This created a local base of healthy ecosystems which greatly facilitated economic growth not only within the forest community but beyond the community through the trade of products. This has ensured that local biodiversity products are marketable.

d. Reduced inequalities and social inclusion

The management of home gardens was not just the duty of men, but an all-inclusive approach by all participants especially women and youths. The selection and maintenance of plant species in the home gardens was influenced by farmers' household needs for food and income, and their knowledge and innovation, which was largely controlled by women and youths. However, in most cases, this was achieved as a result of collaborative efforts among all participants.

Project drawbacks

Developments in plant biotechnology, especially those connected to *in vitro* culture and molecular biology, have provided powerful tools to support and increase the conservation and management of plant diversity (Withers, 1995). These tools have been used to conserve endangered, rare, crop ornamental, medicinal and forest species, allowing for the conservation of pathogen-free material,

A youth in his home garden with *Gongronema latifolium*

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elite plants and genetic diversity in the short, medium and long-term. Thus, they offer tremendous opportunities for improving the well-being of current and future generations and the environment.

Sadly, the public debate on biotechnology is embroiled in controversy over the risks and benefits linked with this emerging technology. Public disapproval of genetic engineering tends to dwarf the known benefits of other biotechnologies, including some that have no direct bearing on gene manipulation, such as plant tissue culture, genomics and marker-assisted selection. This was seen as a major setback during the inception stages of the Umuanunu-Obinze project, as many community members felt that tissue culture plants resulted in modification of the plants to add new traits. There was also evidence indicating a lack of trust.

To mitigate this, the project developed a communication strategy to build trust among community members. Focus groups and follow-up discussion methods were used to build communication trust, key to achieving acceptance of tissue culture plantlets by the community. Another key strategy was to emphasize "local ownership". In all activities, the community was encouraged to "own" the project. Setting up spaces for dialogue that allowed for the active and inclusive participation of local people ensured that priorities were determined locally and that local concerns were at the core of all project activities.

Again, in order to build community confidence, the project was able to build on the community's traditional knowledge of science and technology. Learning from traditional knowledge, by considering what local communities know and have, can increase our understanding of local conditions. It also offers activities designed to help the communities in a productive situation (Warren, 1992). Understanding traditional knowledge further increases the response of communities to developmental projects. The acceptability of tissue culture plants for their home gardens was mainly

Focus group discussions at the beginning (left) and end (right) of the project

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due to the community's local understanding of the functions of home gardens which they have also practiced over decades for other fruit and vegetable species. Adapting science and technology (biotechnology) practices to a local community helped advance the impact and sustainability of community development.

Conclusion

Home garden systems have proven to be avenues for food production and buffers for forest communities via a broad spectrum of providing important ecosystem services. Being part of the agriculture and food production systems in many developing countries, they contribute widely to remedies that will alleviate hunger and malnutrition in the face of the current global food crisis as well as poverty alleviation. The Umuanunu-Obinze tissue culture (micropropagation) project successfully increased the availability of *Gnetum africanum* and *Gongronema latifolium* plants in the community, reduced their exploitation in the forest and increased people's access to nutrient-rich food. The community was also able to maximize the potential of these plants in terms of market and sales, making tissue culture a major contributor to sustainable development, ecosystem restoration and poverty alleviation.

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3.7 Use of DNA tools for identifying timber tree species and verifying the geographic origin of wood in India

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Introduction

In India, the commercial harvesting of timber from natural forests is restricted by the national forest policies to conserve forest resources (Government of India, 1952, 1988, 2018). The huge market demand and inadequate domestic wood supply eventually forced the country to depend heavily on imported timbers and 30 percent of the demand is met through imports (Lawson, 2014). The import of logs into India is regulated by the Directorate of Plant Protection, Quarantine and Storage under the Ministry of Agriculture, Cooperation and Farmers Welfare. Currently, there are no legal mechanisms to exclude illegal timber imported to the country and India, together with China and Viet Nam, has become one of the three largest importers of illegally sourced woods (Kleinschmit *et al.*, 2016; Kollert and Walotek, 2015; Lee, 2018).

Simultaneously, the shortage of domestic timbers has prompted the illegal harvesting and sale of highly prized tropical timbers from the reserved forests, as well as market adulteration with inferior quality timbers that look similar. Difficulties in verifying the species identity and geographical origin of wood with traditional tools like wood anatomy and dendrochronology (study of tree rings), allowed fraudulent cases go unnoticed.

The culprits involved in the crime often escape legal consequences owing to the lack of accurate tools to prove the authenticity (Dormontt *et al.*, 2015). Hence, there is a need to develop a good forensic tool that can provide scientific evidence to the judiciary to convict the culprits and eventually curb the illicit felling of timber species (Dormontt *et al.*, 2015; Dormontt and Lowe, 2019; Jardine, 2020). For this purpose, recently, in addition to wood anatomical and chemical methods, genetic tools have also been incorporated in the United Nations Office on Drugs and Crime (UNODC) forensic timber identification guidelines (UNODC, 2016) and the Global Timber Tracking Network data analysis guide (Gasson *et al.*, 2021).

The unique characteristics of anatomical and chemical methods tend to vary with age and the environment of the standing trees (Parmentier *et al.*, 2013; Nithaniyal *et al.*, 2014). However, genetic methods on the other hand are stable and provide information on species identity, geographic origin and also perform DNA match testing at the individual level. These genetic methods include use of DNA barcodes (short stretches of gene sequences), microsatellite markers (also known as simple sequence repeat [SSR] markers) and single nucleotide polymorphisms (SNPs).

Figure 1. DNA barcodes generated via BOLD for five commercially important
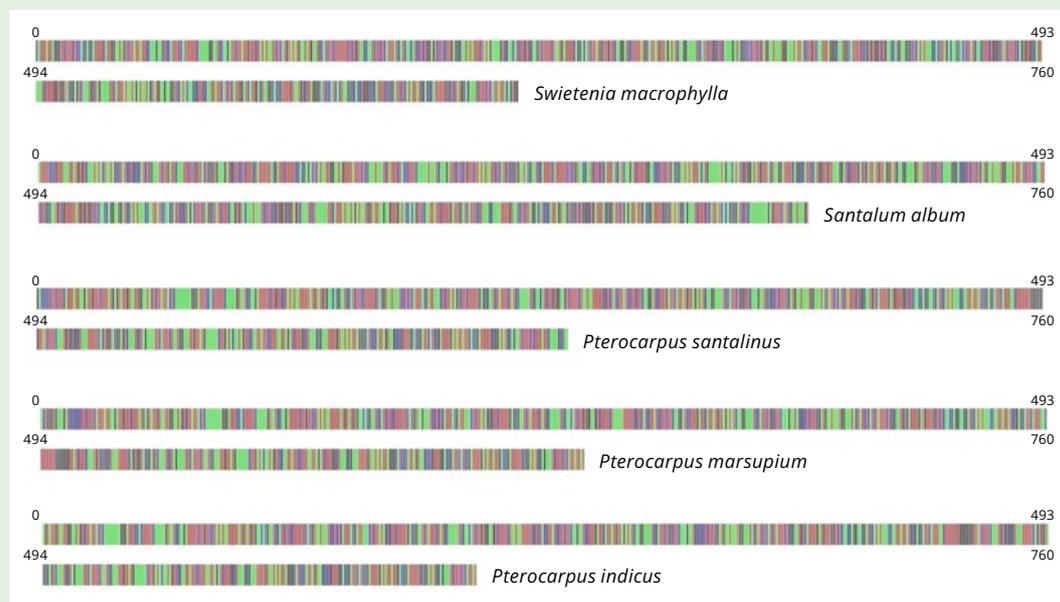
DNA barcodes are specific to each species, like universal product codes (UPC) specific to consumer products, and can be used to confirm the species identity (Hebert *et al.*, 2003; Asif and Cannon 2005; Kress *et al.*, 2005; Fuji, 2007; Degen and Fladung, 2007; Lowe, Wong and Tiong, 2010; Fatima, Srivastava and Somashekar, 2019). Microsatellites/SNPs are DNA tools that are used to differentiate individual trees, basically like a DNA fingerprint in paternity testing. DNA testing in illegal timber felling cases can be done by matching DNA fingerprints developed from original stump (source wood) of the felled trees and seized logs (confiscated by the police) (Dormontt *et al.*, 2015). Individuals from different geographical areas show variation in DNA profile, thus unique DNA profiles specific to each geographic origin are generated (Lowe and Cross, 2011; Vlam *et al.*, 2018). A reference database of DNA fingerprints representing different geographic locations developed in the country is in place to verify the claims of legitimacy made on official documents with regard to origin of the timber. Thus, the logs in a supply chain can be traced back to their source population using the DNA fingerprint pattern.

Species identification: DNA barcoding of 41 timber species in India

Commercially traded timber species are often adulterated in the Indian market with cheap inferior quality species, which mimic the high-value timber species. It is extremely difficult to confirm the species identity based on wood using traditional methods. Major conflicts between the seller and buyer regarding the authenticity of traded wood often lead to the investigation of fraudulent claims. DNA barcodes developed for each species (some examples are shown in Figure 1) are unique tamperproof biological tags (like the UPC barcodes in supermarket products) and have the advantage of confirming the species identity even from processed wood pieces, where traditional tools fail to perform.

In this context, we developed a DNA barcode reference database of 41 commonly traded timber species in south India, including 10 imported timber species in the market (chipped/sawn as well as processed wood). In the first phase, taxonomically authenticated timber species' biological reference material (BRM) were collected from the wild and a DNA barcode reference database was developed adopting standard protocols. In the second phase, these DNA barcode reference databases were used for validating wood samples collected from various sawmills and depots to investigate the authenticity of the claims proposed by the sellers. The DNA barcode sequence correlation analysis could identify disparities in species claims that were made (Degen and Fladung, 2007; Lowe, Wong and Tiong, 2010; Dormontt *et al.*, 2015). We also employed a computer-assisted artificial intelligence platform, called WEKA, for DNA barcode sequence analysis which could add more precision, speed and accuracy to the entire identification process (Hartvig *et al.*, 2015; He *et al.*, 2018, 2019). This approach showed 100 percent accuracy in authenticating the species of traded wood samples.

In India, wood is an integral part of every household, irrespective of their socioeconomic class. Furniture, doors and windows are made from highly sought after timbers like teak, mahogany and rosewood. The developed DNA database was used to address the issues faced by the stakeholders (sawmill and furniture industries) as well as consumers. Both untreated (directly from sawmills) as well as treated logs (for furniture, door/windows making) were used to confirm the identity. Similarly, species authentication of wood sources was also undertaken for the paper and pulpwood industries with developed DNA barcodes in hand. From the analyses performed, cases of misrepresentations and fraudulent claims made by the wood suppliers were identified. The DNA-based timber identification process thus helped the stakeholders to authenticate the wood samples prior to processing for quality furniture without compromising the cost and trust of the customers.

Verifying the geographical origin of wood: an example of teakwood

Teak is one of the highly valued tropical timber species worldwide. In India, teak is considered as an important tree species for its durability and aesthetics. Due to its excellent wood properties, Indian teak is priced highly in the global timber market and exported either as sawn and chipped wood or mostly in the form of value-added products (Midgley *et al.*, 2015; Balakrishnan *et al.*, 2022). Wood properties of teak within India vary according to its geographic origin as environmental factors play a key role in determining the teak wood characteristics including its colour (Nocetti *et al.*, 2011; Moya and Calvo-Alvarado, 2012).

Recently, one of the good quality teak provenances in India (Nilambur teak, from Kerala State) also obtained the geographical indication (GI) tag. In India, GI registration is governed by the Geographical Indications of Goods (Registration and Protection) Act, 1999, indicating the uniqueness of Indian teak. Consequently, the wood of Nilambur teak is priced higher, prompting fake claims from other geographical areas that their wood is 'Nilambur teak' to gain higher prices. Investigation of false claims of origin and mitigation of illegal extraction from reserved forests in the country, require a precise forensic DNA tool. We developed an SSR-based reference database for different teak populations in India that would serve as an authentication database to confirm the origin of teakwood.

Figure 2. Source wood (logs) and evidence wood (stump) identified from a teak plantation logging site in India, used to validate the SSR markers. Markings in the logs and stump during initial felling includes the unique identifier code, grade and quality of the timber harvested

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Development/validation of a DNA (SSR) database for identifying the geographical origin of teakwood

We developed a DNA fingerprint reference database for 425 teak trees representing 18 natural populations covering three gene-ecological zones in India. This reference database was evaluated for its efficiency to assign individual teak trees in reserve forests to their respective geographical zones. DNA fingerprints of unknown teakwood were employed to identify the source population using the reference database, with an artificial intelligence based analytical platform (AssignPop).

To begin with, validation of the database was carried out in a blind test framework (log and stump recoded to provide no information as to which log belonged to which stump). We collected stumps and logs (Figure 2) from plantations under clear felling (Figure 3) and generated their DNA profiles which clearly distinguished the logs from one another. The genetic profiles of source wood and seized wood showed 100 percent similarity. Likewise, origin verification of 70 individual trees sampled randomly from three different natural populations, when tested using the reference database showed high assignment efficiency (Figure 4). Furthermore, these markers were also used to verify the integrity of the supply chain (logging site to the point of sale) by comparing the genetic profile of randomly chosen logs in a lot (minimum 10 percent) and confirming their source.

The developed database was also used to test the mixed origin of teak plantations in the country which were established from seeds of diverse natural populations. Along with similar databases generated from other teak growing countries, it can be conjointly used to authenticate and verify the origin of traded teak logs (as claimed in their accompanying documents) in the global timber market.

Figure 3. A 60 year-old teak plantation at Malayatoor, Kerala, India under clear felling

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Case studies on DNA testing to combat illegal extraction

The DNA fingerprint database was employed to trace the origin of wood at individual levels as well as to match the seized wood (log) and source wood (stump) seized by the Forest Department by matching the genetic profiles of the logs with the stumps. DNA evidence was provided to judiciary in more than five registered cases related to illegal felling of timber in Kerala State, India. This fingerprinting tool has immensely benefited the Kerala State Forest Department to produce scientific evidence and take legal actions against the culprits. The technology guarantees confidence to small scale plantation holders for the desired productivity by verifying the genuineness of the claimed planting materials of superior origin (e.g. Nilambur teak) during the time of establishment of plantations.

Summary

The quantity of illegal timber reaching India, along with disparities in the documentation and permits produced, essentially recommend for a strong regulation to be in place. In 2018, the Government of India approved "Vriksh", a timber legality assessment and verification standard developed by the Export Promotion Council for Handicrafts where the legality of the wood is verified by checking the documents passed through various owners along the supply chain. Though the organization considers geographic origin to be a major criterion to verify legality, document verification is the

Figure 4. Assignment of unknown individual trees randomly sampled from natural populations (70 individuals, three populations) to their source populations using a reference database (18 populations, 425 individuals) with a machine learning approach. The image, generated using the software AssignPop, shows a 100 % accuracy in the prediction of unknown individuals to their source population. X-axis: individual test samples with unknown origin and Y-axis: posterior probability in a range of 0-1. Reference populations (abbreviated as ID, KON, WY, NIL, ARK, PAR, TH, TEN, ACH, KMTR, STR, ATR, TEL, SHI, TTM, HAL, CU and HBD) labelled from 1-18 are indicated in specific colours. The origin of unknown individuals is predicted by the colour of the population to which they have been assigned - 22 individuals labelled ARK (Aryankavu), 26 labelled as NIL (Nilambur) and 22 labelled PAR (Parambikulam)
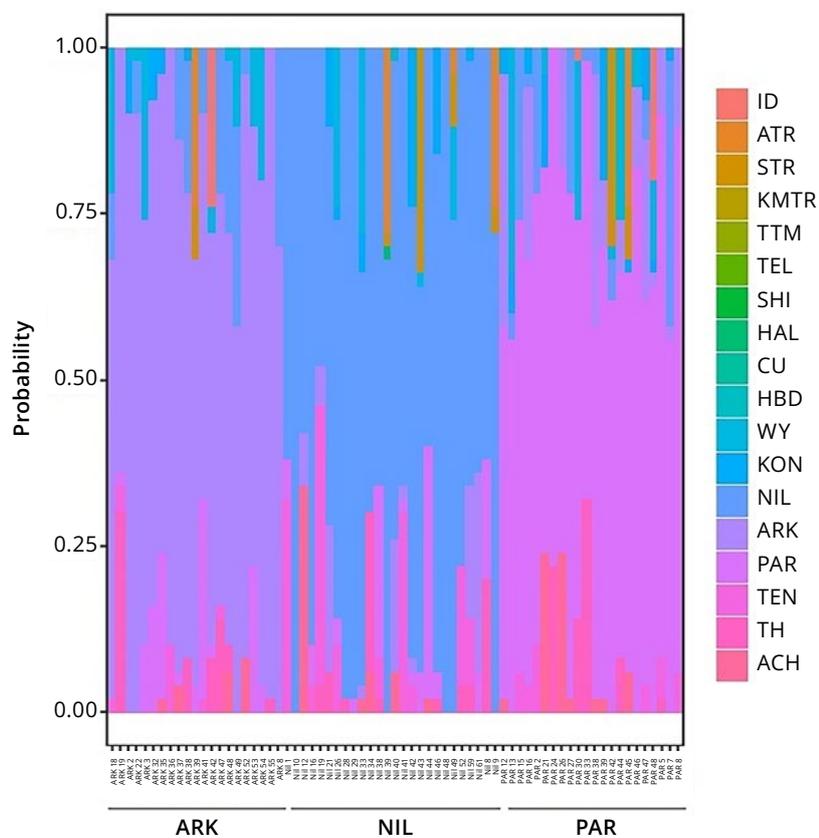
only method utilized and logs with no information on their origin are set aside until clarification is made. The situation thus warrants a good forensic tool for proper assessment and precision.

In this context, our study demonstrated the utility of DNA forensic tools (DNA barcodes/DNA fingerprints) to validate and verify the species' identity and geographical origin of the traded timbers along the supply chain. The study also proved the efficacy of these DNA tools through the case studies undertaken to provide scientific evidence to the judiciary. Thus, the information generated from our study helped the Forest Departments as well as various stakeholders (small scale plantation holders, timber mill owners, paper/pulp and other industrialists) to cease conflicts over the authenticity of the value-added timber-based market products and to assess the legality of the traded timber.

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3.8 Teak tissue culture to encourage farm forestry in India

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Introduction

Teak (*Tectona grandis* L.f) is one of the most valuable timber tree species because of its unique blend of characteristics with unrivalled quality. The wood of Indian teak is characterized by straight or wavy grains with mottles, an oily surface marked with a white sheen. Teak can be considered a climate-friendly alternative, substituting energy-intensive materials. Teak-planted soils also hold significant amounts of carbon on par with natural forests (Sasidharan and Ramasamy, 2021).

Teak has been the most favoured species for tree plantations in the tropics for centuries. Its global plantation area has crossed 6.8 million ha, of which 80 percent is in Asia. More than 90 percent of the world's teak resources are found in Asia (Kollert and Kleine, eds, 2017) and the global teak market is led by trends in its Asian market. India, followed by China, receives the majority of the world's teak trade flows. Collectively, these two nations account for more than two thirds of teak traded, raising the mean global annual trade to more than one million m^3. Despite occupying the largest area of natural teak forests, and the highest levels of genetic diversity (Prasetyo *et al.*, 2020), India is the world's second-largest importer of the species.

The first commercial teak plantation in the world was established in 1842 in Nilambur, India, and more recently foresters and farmers identified teak as a potential species for domestication (e.g. Pachas *et al.*, 2019). It is also popular in agroforestry because of its rapid growth in the early years and standardised management practices (Mohan Kumar, 2007; Dhyani *et al.*, 2021). Plantations where trees are harvested at an older age (i.e. long rotation plantations, 50–60 years) produce large-sized logs. They fetch high market prices, equivalent to natural forest logs. But this involves decades – a deterrent to investments.

Teak has a promising future in India as a component of agroforestry. One of the causes of uninterest among farmers in teak cultivation in India is the exaggerated output projections of a few commercial tree investment programmes by private players, leading to its downfall (Balooni, 2000). Recent studies in India estimate a productivity of 180–190 m^3/ha in block plantations that are 15 years old with a density of 2 000 trees (Shahapurmath *et al.*, 2016). An unmanaged 12-year-old block plantation showed 140–150 m^3/ha, with a density of 2 000 trees (Shukla and Viswanath, 2014).

Shorter rotation periods are being increasingly practised worldwide to meet the growing demands and to support teak growers. Short-rotation teak plantations initiated in Thailand during the 1990s (Mittelman, 2000) and have gained the attention of many tropical countries. At the same time, teak clonal forestry has demonstrated its efficiency for short rotation plantations worldwide.

Currently, most teak plantations in tropical and subtropical countries are raised from tissue culture-derived planting stock (Monteuuis and Maître, 2007; Monteuuis and Goh, 2018). The visionary commercialisation of a handful of elite teak clones from a Malaysian laboratory (Goh *et al.*, 2007; Monteuuis, Bon and Goh, 1998) brought the potential of cloning teak into reality, setting a new benchmark for genetic gain globally.

Despite systematic teak genetic improvement programmes since the 1960s, the development of vegetative propagation techniques and generation of genome-level information (Kedharnath and Matthews, 1962; Kumaravelu, 1993; Yasodha *et al.*, 2018), integration of advanced technologies for breeding is yet to be achieved.

Though India has had a lead in developing tissue culture protocols in teak since the 1970s, commercial scale realisation is disappointing. The Government of India realized that the only option was to intensify research and increase interaction between industries and research labs. The Department of Biotechnology initiated a major project in 1989–1990 for producing tissue-cultured tree species stating that seeds of uncertain genetic quality, when used for planting, would lock lands for long periods under inferior, low-yielding plantations.

Around 200 commercial tissue culture labs with a production capacity of approximately 350 million plants exist in India. Teak, however, occupies a very insignificant position mainly due to a lack of motivation to adopt tissue culture technology, insufficient field demonstration trials, inadequate data on the performance of clonal planting stock and the high cost of plants. Sporadic field trials of tissue culture raised teak are inadequate to convince the tree growers to establish new plantations. Commercial production and establishment of teak plantations is feasible with public-private partnerships, considering the large number of private tissue culture labs in India.

To complement the available technologies for mass propagation of genetically improved stock, and to ensure the perpetuation of the superior germplasm, tissue culture technology using explants from genetically superior trees can be one of the viable options (Kendurkar, 2011). There is a need to enhance the per unit area productivity instead of extending the plantation area (Goh and Monteuuis, 2005).

Protocols for tissue culture of teak

The Institute of Forest Genetics and Tree Breeding (IFGTB) at Coimbatore focuses on genetic improvement and clonal production of teak through tissue culture. The IFGTB is engaged in establishing base populations, clonal selections, and evaluating and identifying genetic diversity hotspots with particular reference to Tamil Nadu, Kerala and Karnataka. The selection of genotypes offers variations and exhibits fast growth. Individual tree selections based on the growth rate, stem form, clear stem height, branch angle, and pest and disease resistance were deployed as stock plants for tissue culture. The IFGTB is developing descriptors for registration of teak clones with the Protection of Plant Varieties and Farmers' Rights Authority, Government of India, to secure intellectual property rights.

IFGTB developed a micropropagation protocol for teak during 1995–2001 with support from the World Bank funded project on Forestry Research, Education and Extension (World Bank, 2002). However, certain problems were encountered, including the length of time needed for culture establishment, low frequency of shoot production and two-step methods for *in vitro* rooting procedures. Nodes

Figure 1. Height (m) of different teak clones and seedling in a study by the IFGTB (year of planting: 2000). MHAL – Maharashtra clone, SBL – Andhra Pradesh clone, TNT- Tamil Nadu clone
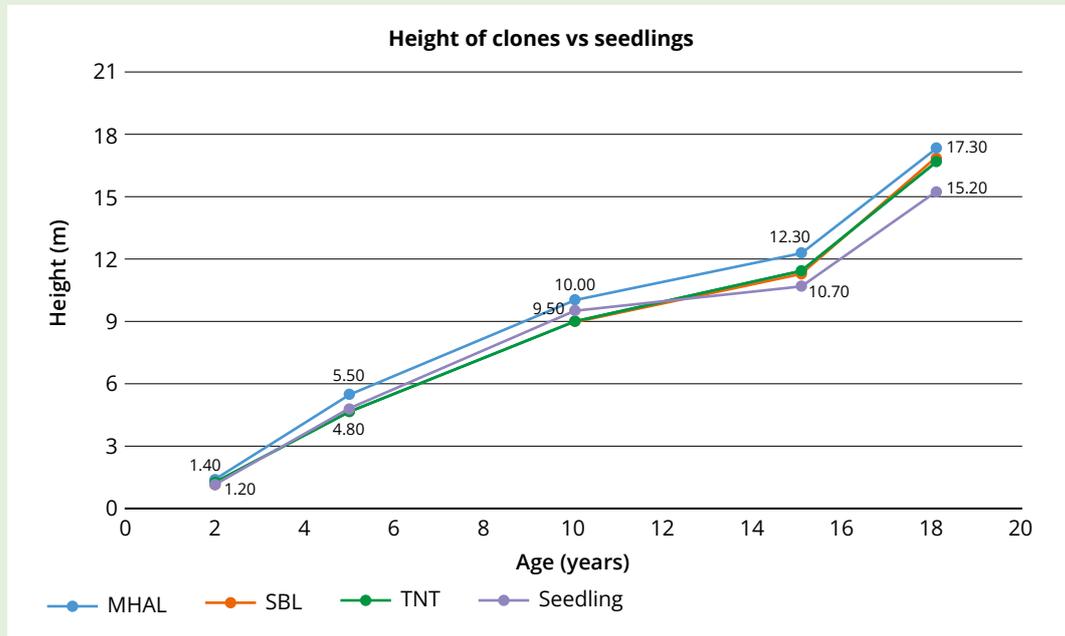
or shoot apices are induced to produce multiple shoots followed by root induction either under *in vitro* or *ex vitro* conditions. Cultures are most readily obtained with juvenile explants derived from seedlings, from shoots at the base of trees or from the shoots produced by repeated grafting of buds from mature trees onto seedlings. The complete removal of the bark from around the entire circumference at the base of the trunk triggers the growth of juvenile shoots. Actively growing tips obtained during bud break are also amenable to rejuvenation.

Procedures for shoot multiplication were optimised after testing various nutrient media, growth hormones, solidifying agents, different light conditions, subculture cycles and subculturing methods to increase the number of multiple shoots produced. Genetic fidelity of the tissue culture raised plantlets ensures conformity with the mother plants. Accordingly, simple sequence repeat (SSR) markers were developed and used to ensure genetic fidelity and uniformity. After-care of *ex vitro* rooted shoots to enhance survival rates, including fertilizer application at various stages of the rooting-acclimatisation phase, was established to ensure better survival on outplanting.

The IFGTB established a field trial comprising three clones with seedlings as a control in the field research station in Kerala. The details on performance of the clones in comparison with seedlings is presented in Figures 1 and 2. Figure 1 shows that as the years passed the clones were taller than trees which developed from seedlings. Figure 2 shows that the clones were considerably thicker than trees grown from seedlings. This was followed by projects from state departments – the Forest Development Corporation of Maharashtra and Kerala Forest Department – to assess the growth performance of tissue culture plants in different locations.

Figure 2. Diameter (cm) of different teak clones and seedling in a study by the IFGTB (year of planting: 2000). MHAL – Maharashtra clone, SBL – Andhra Pradesh clone, TNT- Tamil Nadu clone
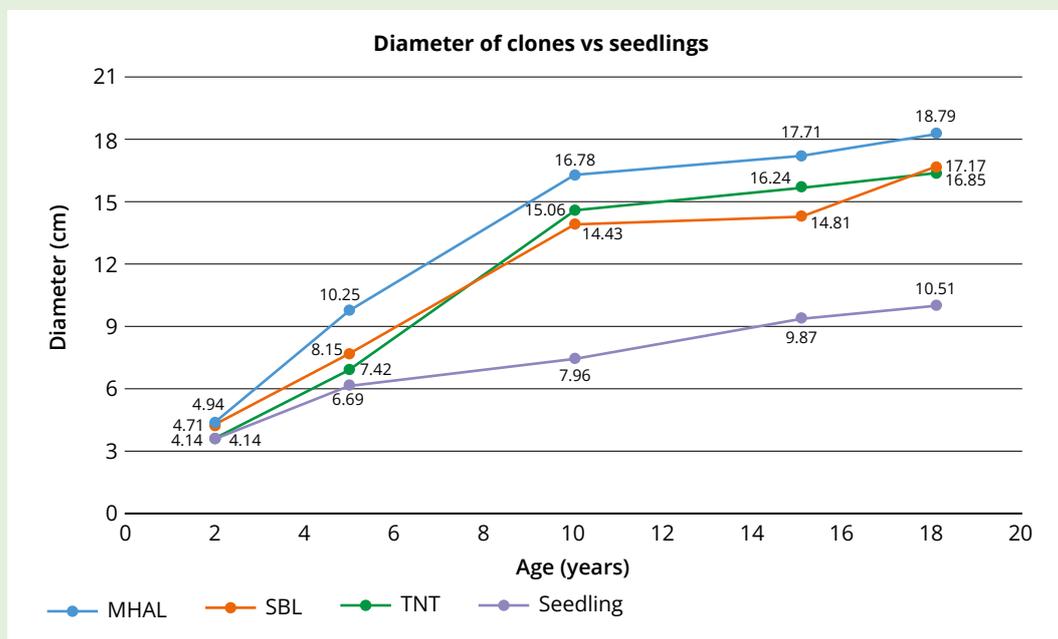
A national programme on teak cloning

Recognizing the scope of this technology, the Indian Ministry of Environment, Forest and Climate Change (MoEFCC), through the Indian Council of Forestry Research and Education (ICFRE), formally launched a national programme in 2019–20 on teak cloning to popularize small scale clonal plantations across the country. This All India Coordinated Research Project on Biotechnological Interventions in Tree Improvement – Quality Teak Production: Capitalising on Cloning of the ICFRE is an extension of biotechnology to the field (ICFRE, 2021). The IFGTB has initiated large-scale multiplication of clonal selections from its breeding programmes and is supplying tissue culture teak plants to farmers in Tamil Nadu, Karnataka, Odisha and Chhattisgarh at highly competitive prices. The project also aims to enrich the teak genetic resources by deploying regionally available material, thereby addressing the issue of adaptability across locations.

The IFGTB provided hands-on training and start-up cultures of teak (five clones) to the four participating ICFRE institutes. Starter cultures of the five clones of teak are presently being multiplied at all the institutes. The quality planting material thus produced is distributed to farmers to popularise tissue culture teak. Convinced with the fast growth of the plants, farmers have evinced interest in increasing the area under teak. Since 2021, the IFGTB has collaborated with commercial labs to mass-produce teak. In 2022, the production target is 1 million plants to meet the increasing demand. Agreements have been signed with five commercial tissue culture labs to mass-produce teak to meet the increasing demand.

Initial obstacles and challenges had to be continuously addressed, but the growing success strengthened the institutional desire to continue the efforts. The increasing interest of farmers,

Figure 3. Distribution of teak plants to different states from 2015 to 2022. A total of 362 500 plants were supplied during the period
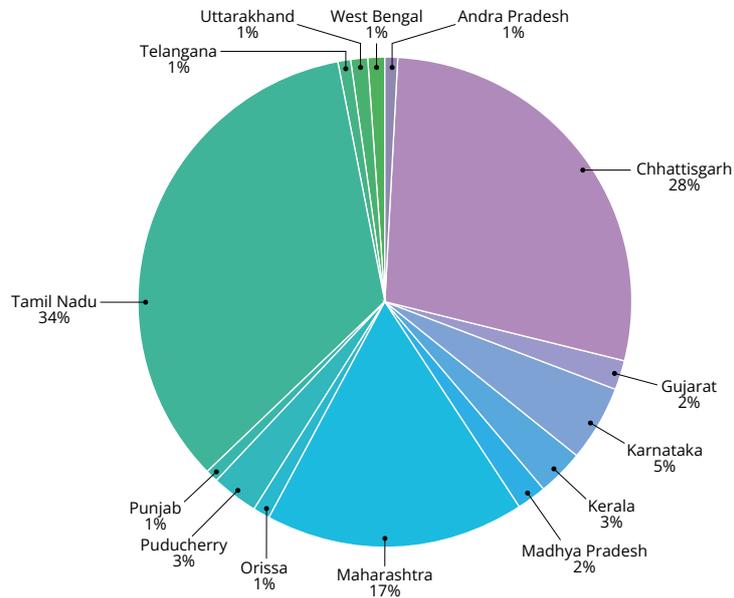
who experienced the fast growth performance of the quality planting material, intensified, leading to further demand (Figure 3).

Though the protocol was standardized early on, large-scale production was initiated in 2016. The IFGTB supplied about 0.36 million plants to farmers for block and line plantations. Farmers follow the management and irrigation regimes prescribed by the IFGTB. Initial results are encouraging as the plants exhibit fast growth in well-maintained farm fields.

About one hundred farmers of this group (roughly 70 percent) have small land holdings. They manage about 0.5–3.0 ha land with an average of 1.0 ha. The species is grown as pure stands, mixed plantations with fast-growing multipurpose trees or boundary plantings along with annual crops. Most farmers prefer to grow annual crops in the stands, when the trees are young, This system is viewed as a long-term investment – a live bank account, and ensures an additional income, since the species' demand in the market continues to grow. The farmers also state that the intention to grow short rotation teak is to support the local demands rather than exploring the market opportunities.

There is a growing demand for teak clones in the country. State forest departments and farmers show strong interest in raising large-scale clonal plantations. The current growth of these plantations has a harvest potential of 15–25 years. Site conditions and management practices are crucial in deciding the harvest age.

Given the initial challenges, the main success factors were that the forest departments of teak-growing states supported the project and involved various stakeholders such as farmer associations,

**Figure 4. An upward trend is observed in the demand of tissue culture raised
teak plants since 2016 to the present**
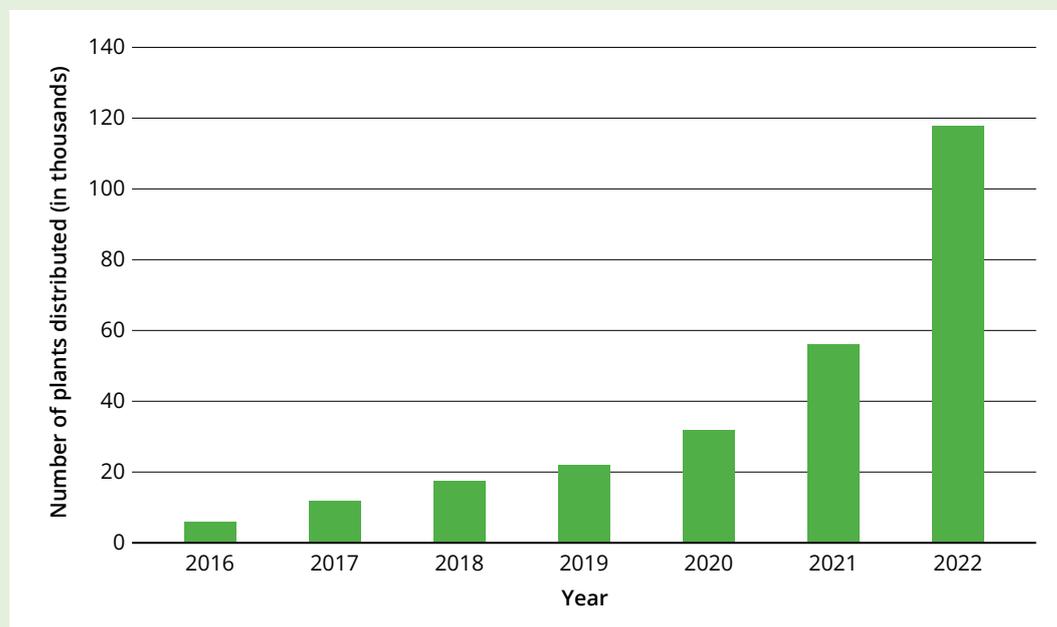
NGOs, the private sector and academia. The IFGTB continues to benefit from the support of the ICFRE and MoEFCC for technology transfer and capacity development.

Based on research results showing increased growth rates and shorter harvest ages, it is expected that the clonal teak can contribute significantly to the exchequer compared to conventional propagation methods. There is a continuously increasing demand for teak (Figure 4). Indeed, during April to September 2022, 75 000 plants were sold to a single state.

Training on tissue culture

In line with the Skill India Mission, the MoEFCC has launched a Green Skill Development Programme under which a training programme on "Plant tissue culture techniques and its applications" is supported. The course aims to provide specialised technical skills in plant tissue culture for a successful career in entrepreneurship and generate technically trained human resources for tissue culture industries. The training programme is for 40 working days (340 hours). The course is outlined to give a unique learning experience through focused interactions in the classroom and hands-on exercises comprising 70 hours of theory and 270 hours of practical work. The participants are also trained on issues like quality assurance and quality control, the analysis of risk factors and cost-benefit analysis, banking linkages and entrepreneurship, problems associated with tree tissue culture, and scope of tissue culture for species in demand like teak. Five batches have been trained since 2019, and 40 percent have started their own labs/nurseries.

Conclusion

Teak represents a good livelihood opportunity for tree growers and is a major asset for the forest sector and the whole economy. The long-term prospects for plantation-grown teak appear promising, and demand will likely increase in the future. Investments in teak plantations growing under suitable site conditions with genetically superior planting material and suitable silvicultural and management practices can yield attractive and robust financial returns.

Acknowledgement

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Performance of tissue cultured teak in farms at various stages of growth (6 months, 1 year, 1.5 years, 2 years, 2.5 years and 5 years). The plants have been established in farmers' fields since 2016

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Five-year old tissue cultured teak planted along the boundary of a field in Salem, India. The farmer is on the left and two IFGTB staff are on the right

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Six-month old tissue cultured teak planted with groundnut as an intercrop, with the farmer in his field in Puducherry, India

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Teak field grown with groundnut as an intercrop after 4 years. This is the same field as the previous photo 4 years later

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Tissue cultured teak intercropped with banana, casuarina (a nitrogen-fixing pulpwood species) and *Melia dubia* (a plywood species) with the farmer in his field in Tindivanam, India

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A five-year old block plantation of tissue cultured teak in a farmer's field
in Narharpur, Raipur, India, with progressive farmers in the field

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3.9 Applications of biotechnologies to rescue the biological and cultural heritage of the Patagonian people: the case of *Salix humboldtiana*

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The species

The evolutionary units of a species, that is its populations, may be threatened at different levels prior to their local extinction. The most serious situation occurs when the level of "erosion of knowledge" is reached, when the inhabitants of a region no longer know the species that originally occupied that socio-cultural space. This occurs in the Argentine Patagonia with the only native willow of Latin America, *Salix humboldtiana*, which can be considered one of the most threatened tree species of the country.

It is one of the tree species with the longest natural distribution area in the world. It spreads over 10 000 km from central-north Mexico (23° N) to Chubut River (44° S) in the Argentine Patagonia. As a riparian species, it grows in a great diversity of environmental conditions. In Argentina it is widely distributed, being present in almost the whole country except for the high altitudes of the Andes and south Patagonia (Gallo *et al.*, 2020).

Salix humboldtiana is a medium-sized tree, growing up to 25 m in height and up to 1 m in diameter. It is a pioneer species and can tolerate long periods of flooding, up to seven months with submerged roots. It is frost sensitive, which leads to some range restrictions in the cold environments like those of high altitudes and the South Patagonian steppe (Gallo *et al.*, 2020).

With 20 to 25 million years of evolution, it is an ancient and phylogenetically basal species of the genus, and probably the origin of many other *Salix* species. Most of the willows are polyploids and of much more recent species origin (of about 3–5 million years) whereas *S. humboldtiana* is diploid. Its evolutionary origin is in the subtropical New World and, from there, it migrated to the southern part of South America, adapting to low temperatures.

It regenerates mainly through seeds dispersed by wind or water, and commonly builds sapling stands on sand or lime banks. It has very narrow leaves (0.6–0.8 mm) that are not very long (6–13 cm), some of them with a slight curve and slightly sawed. It is an evergreen species when it occurs in warm tropical and subtropical climate conditions, but deciduous in temperate and cold environments or during dry seasons. The main adult trunk shows a rough bark with irregular plates, and its architecture is very characteristic showing a monopodic growth with very straight trunks up to 7 m height (Figures 1 and 2).

Figure 1. Three *Salix humboldtiana* natural individuals in San Javier population (Río Negro Province). Their forest shape stands out in comparison to introduced willows in the back

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Figure 2. Typical bark of *S. humboldtiana*

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The main causes of genetic diversity loss

Salix humboldtiana has been used since at least 10 000 years ago by Indigenous Peoples to make different tool handles. It has an excellent wood quality of reddish color and an average basic density (0.50 g/cm^3) superior to that of all other willows introduced in Argentina. The quality of its wood is so good that the Indigenous Peoples trusted it even to manufacture elements of vital importance in their daily lives such as arrows and spears. European settlers cut the trees down, leading to its almost total devastation. Its natural regeneration by seeds and its inability to regrow from low stumps contributed to its decline. Nowadays, the very few remnant trees are still being cut down clandestinely.

In most of the rivers of the country, the natural distribution is highly fragmented. Several historical registers confirm population's extinctions (Entraigas, 1960) in most of the Patagonian rivers. One of the main causes of the critical loss of its genetic diversity in the last 250 years was the massive use of its wood during the European colonization of all rivers, crossing arid and semi-arid regions (60 percent of the country) and the very fertile Pampas, where no other wood of such quality was found. It has been mentioned that thousands of trees were cut down per week by the European settlers to build the first villages (houses, fortifications, churches, fences and even boats) (Entraigas, 1960).

The introduction of highly vigorous and invasive exotic *Salix* clones, initiated some 150 years ago, has further affected the remaining natural stands. Their higher vegetative propagation capacity contributed to the reduction of the native willow habitat.

Salix x rubens is the natural invasive hybrid between *Salix alba L.* and *Salix euxina* I. *(ex S. fragilis)*. A study with molecular markers determined that along the Patagonian rivers most of the willow trees (93 percent) belong to the same and only female clone of *Salix x rubens* (Budde *et al.*, 2011). In a greenhouse experiment, an inferior vegetative propagation capacity of *Salix humboldtiana* was confirmed when compared with introduced *Salix* clones (*S. fragilis var. fragilis, S. babylonica* hybrid and *Populus* spp.) under different humidity conditions (Thomas *et al.*, 2012).

Identification of interspecific hybrids

To assess the genetic diversity of *S. humboldtiana* and to unravel the population genetic structure along the Patagonian rivers, samples were collected in 34 locations, most of them along the Río Negro river. By genotyping at five nuclear microsatellites (Bozzi *et al.*, 2015), a lower genetic diversity was found (Ho = 0.568; He = 0.555) in *S. humboldtiana* compared with other riparian tree species. A trend of increasing genetic diversity was observed downstream along the Río Negro. Other research showed that a genetic diversity cline is present in a north-south direction with less variation in the southern Patagonian rivers (Pomponio *et al.,* 2018).

The huge invasion of all river shores by exotic willow clones has greatly contributed to the hybridization process given the high demographic imbalance between the hundreds of thousands of exotic *Salix* trees and just a few surviving native willows. The probability that an insect carrying native willow pollen fertilizes a native female tree is very small. On the contrary, the probability of fertilizing a female exotic willow clone is very high. Therefore, in most of the rivers, when the reproductive cycles of the native and exotic willows overlap, hybrid seeds are produced. Backcrosses again take place only with the exotic clones.

Figure 3. a) Electropherogram of one pure individual of *Salix humboldtiana* in locus PGMC 223 showing a homozygote genotype with the diagnostic allele of 176 bp. b) Electropherogram of one hybrid individual of *Salix humboldtiana* in locus PGMC 223 showing a heterozygote genotype with the diagnostic allele and another allele of 194 bp
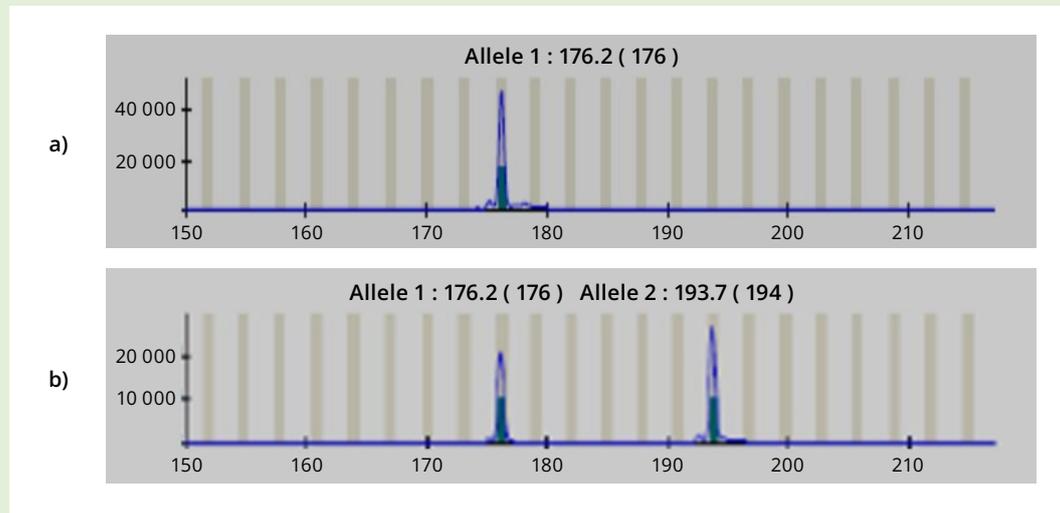
In order to identify the hybridization processes among the invasive willow taxa (*S. alba, S. fragilis, S. babylonica, S. matsudana, S. viminalis)* and *S. humboldtiana,* a molecular toolbox for taxa discrimination was created. It combines three maternally inherited chloroplast microsatellites with ten biparentally inherited nuclear microsatellite markers. This toolbox is suitable to unambiguously distinguish first generation *S. humboldtiana* hybrids. The taxa can be discerned by the chloroplast microsatellite markers and, in addition, *S. humboldtiana* presented an exclusive allele at one of the nuclear microsatellite loci (Figure 3). The analysis of 1300 individuals from five Patagonian rivers, allowed 86 *Salix humboldtiana* hybrids to be detected: 76 percent were juvenile and only 24 percent adults. In most cases, *S. humboldtiana* was the pollen donor, which is expected, since the most distributed exotic willow clone in the study area is female (Budde *et al.*, 2011).

Additionally, a molecular pair comparison was made at 15 sites of the Río Negro, where adult trees (> 20 years) and groups of juveniles (< 5 years) grow together. It was found that the number of populations with hybrids doubled in the last 20 years (50 percent of the juvenile groups analyzed had hybrids) and that the number of hybrid individuals increased three times in the last 20 years (16 percent of the regeneration) (Gallo *et al.*, 2018). The higher proportion of hybrids at the juvenile stage indicates the intensification of the hybridization process.

This means that the few remnant trees of the native species are subjected to the mechanism of introgression (introgressive hybridization) which leads to genetic dilution of the native genetic information.

The participatory rescue programme

In year 2008 we began with a molecular genetic diversity analysis together with the University of Marburg, Germany. During the sampling, we could find just a few surviving *S. humboldtiana* individuals along the Patagonian rivers, indicating that the situation for the species was serious. We therefore decided to begin practical rescue work of the species in 2011, including a set of many field and social activities that ranged from propagation of surviving trees, restoration and social dissemination for awareness.

We began an awareness raising campaign with the inhabitants of the Patagonia region, including the rural communities in small villages. Several meetings with Patagonian communities' authorities were held and many talks in rural schools were given. We talked about the species and showed the phenotypic traits of the trees in order to teach the people how to identify the native species. We explained the historical and abusive destruction of the species leading to the extinction of several populations. We established small forests of the species in frequently used parks and other public spaces, called "knowledge rescue small forests" (Figure 4). Their purpose was to connect people with the native species that covered the rivers in the past with a very dense gallery of continuous forests.

The interest to work with the species spread afterwards to the whole country. Researchers and/or communities have now done restoration and research activities in Tucuman (north of the country), Entre Ríos (central east), Buenos Aires Autonomous City, Buenos Aires Province, etc. All these activities began from the initial work done at IFAB in Bariloche.

The participatory rescue programme had several different stages, including, among others:
1. Locating and mapping the surviving trees along 2500 km of the Patagonian rivers
2. Collection of tree branches, for use in vegetative propagation
3. Propagation at the nursery through a semi-hydroponics method
4. Installation of regeneration diversity forest (restoration of river shores)
5. Clonal tests installation
6. Clonal banks installation
7. Nurseries installation in several different localities
8. Presentations in congresses
9. Presentations in courses about restoration of forest ecosystems
10. Talks in schools, in rural schools, in social events about environment management, in nurseries
11. Interviews for newspapers
12. Interviews for radio and television programmes
13. Interviews with local, provincial and national authorities (mayors, governors, ministers, senior administrators etc.)
14. Publication of brochures
15. Development of internet pages

The idea and first work began in INTA, but after showing our first results we were able to obtain support from the Environmental Ministry, provinces, local communities and inhabitants of villages etc. Research groups of three national universities then began developing their own research work with our help, providing knowledge and material. By the end of the programme more than 35 institutions formed part of it. Among them were nine different INTA institutes, six national universities, two government ministries, three provinces and about 17 localities.

Figure 4. A "Knowledge rescue small forest" in a public park, Viedma City, North Patagonia, Argentina

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We showed our molecular markers results, demonstrating that the biggest current threat is the increasing interspecific hybridization and the consequent loss of *Salix humboldtiana* genetic diversity. This was of decisive importance to motivate people to conserve the species. About ten communities (cities and villages) of Patagonia became aware of the critical situation of the species and joined the programme. Without the use of molecular markers, it would not have been possible to explain and prove the complex population genetics process that led to the participatory rescue programme of the species.

The programme lasted 12 years, from 2010 to 2022, and it had various impacts. Even though the programme recently finished, many of its activities will continue throughout the country. Societal awareness about the species was the main impact of the programme. Just 12 years ago, the species was almost unknown. Not even professionals and researchers in environmental and biological sciences knew about our native willow species.

Through the many interviews that we had with different authorities (such as INTA, CONICET and environmental bodies at the provincial level), we managed to find a place for the native willow in forest tree species prioritization processes at the local, regional and national level.

Because of our work, people now recognize the species in Patagonia and in the whole country. In several localities, people see again the native species that once covered their rivers two hundred years ago. There were also physical impacts of the programme. We work with the species in the field in 17 different sites, encompassing restoration forests, knowledge rescue small forests, clonal tests, clonal banks etc.

All society benefited from this programme, not only in Patagonia, but also throughout the entire country. City people from Patagonia living on the shores of some rivers saw a reappearance of the native willow species they were not familiar with. The general feeling is that part of the local identity has been rescued. Most people are therefore enthusiastic about participating in the field and diffusion activities. There was increased interest in the wood species amongst rural people from Patagonia because of its high quality. There is an awareness of the excellent quality wood and its rapid growth. They were as a result keen to offer a portion of their land for our trials. Local people could also see a future economic benefit from the cultivation of the species. Furthermore, people from other parts of the country have read information about the species and become so interested that we have received orders of plants from them.

We did not face problems in explaining the importance of the use of molecular markers to assess the genetic diversity of the species and to document the ongoing interspecific hybridization process. Our experience of working with rural people and local communities in other projects gave us the possibility to clearly explain the serious situation of our native Salix species and the contribution of molecular markers to taking practical decisions to its conservation.

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Chapter 4: An overview and reflections on the 15 case studies

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Executive Summary

This short chapter provides an overview and a number of reflections on the 15 case studies when considered collectively. Although a modest number, they nevertheless represent a heterogenous collection of case studies, reflecting a good sample of the wide diversity of biotechnology applications which are currently taking place in developing countries. Eight were from Asia, four from sub-Saharan Africa, two from Latin America and one included countries in both sub-Saharan Africa and Asia. They covered applications in buffaloes, cattle, chickpeas, cowpeas, dragon fruit, eggplants, golden berry, maize, Nile tilapia, pigs, rice, soybeans, tree tomato and willow as well as 41 timber species, including teak, and two forest climber species known locally as okazi and utazi.

The case studies all looked at applications of agricultural biotechnologies to meet the challenges facing smallholders in developing countries. These challenges varied, depending on the specific place and context, but there were also many commonalities among them. A common main challenge facing smallholders in several of the case studies was that there was low production and that biotechnologies were therefore applied to increase their yields, while also helping them to face the challenges of climate change, and thereby increase their income. A number of the case studies also described how biotechnologies were used to help smallholders fight against biotic stresses. Four case studies covered aspects related to the characterization and conservation of genetic resources for food and agriculture.

The case studies included a wide range of low- to high-tech biotechnologies and encompassed the use of artificial insemination, DNA barcoding, doubled haploidy, genetic modification, genome-wide selection, induced mutagenesis, marker-assisted selection, micropropagation, microsatellite markers, polymerase chain reaction, semen sexing and the sterile insect technique. In several case studies, two or more biotechnologies were used together.

Regarding obstacles to the use of biotechnologies, about half of the case studies mentioned technical challenges, which can be quite common in scientific endeavours. The limited understanding by the farmers of the biotechnology being applied was also cited as a challenge in some case studies. For the two case studies using genetic modification, some specific challenges related to this technology were also mentioned.

All the case studies attempted in some way to describe the results that have been achieved from applying biotechnologies. The impacts reported in the case studies were consistently positive for

the different indicators considered. The most common indicators of impact discussed in the case studies were of an economic nature. However, in some cases, this was not straightforward as the biotechnology products, such as new crop varieties, had only been made available to smallholder farmers quite recently. Furthermore, for longer living species, such as dairy cattle or teak trees, documentation of impacts requires additional time. For this reason, in a couple of cases, the results of an *ex ante* impact assessment were provided.

In some other case studies, even though the biotechnology products were only released recently, the authors sought to provide some early indications of their economic impacts in the field. In others, the case studies described initiatives that were long-running and where the impacts of the biotechnology products were well-documented. For example, a case study on the use of induced mutagenesis for crop improvement in Viet Nam estimated that the rice mutant variety DT 10 had been cultivated on a cumulative area of 2.4 million ha between 1990 and 2014, benefiting 3 million farmers and increasing their income by USD 450 million.

Many case studies also documented impacts that did not have any direct economic benefit (although they might have indirect economic benefits). These included case studies reporting that the application of biotechnologies had reduced the use of pesticides which, apart from the economic benefits, also had potential positive benefits on the environment and human health. The case studies also showed that biotechnologies were very useful for the characterization and conservation of genetic resources for food and agriculture.

From a collective consideration of all case studies, four key ingredients for the successful application of biotechnologies to meet the needs of smallholders in developing countries were identified. The first, and arguably the most important, was close partnerships between researchers and relevant institutions in developing countries with those in advanced research institutions, CGIAR centres, the private sector and/or UN bodies. The second was long-term sustained commitment, financial and non-financial, from stakeholders in the initiative. The third was political and/or financial support from national and/or local governments. The fourth was good communication with farmers and other relevant stakeholders.

1. Introduction

Reading through the 15 case studies, one thing that strikes the reader is their heterogeneity. This is not surprising, however, as agricultural biotechnologies are essentially a large set of tools and, as such, they can be applied in a very wide range of situations depending on factors such as what and where the problem is and what tool is considered most appropriate to resolve the specific problem.

The 15 case studies reported experiences from different regions of the world. Eight were from Asia, four from sub-Saharan Africa (SSA), two from Latin America and one included countries in both SSA and Asia. Twelve case studies were based in a single country, namely Argentina, Bangladesh (two case studies), Ecuador, India (three case studies), Madagascar, Malawi, Nigeria, the Philippines and Viet Nam. Three covered more than a single country, namely Malaysia, the Philippines and Viet Nam (case study [CS] 2.6), Ethiopia, India, Kenya and the United Republic of Tanzania (CS 3.4) and 14 different SSA countries (CS 3.5).

The case studies encompassed a wide range of different biological systems. For the animal kingdom, they covered applications of biotechnologies to buffaloes, cattle, Nile tilapia and pigs. For the plant

kingdom, they covered chickpeas, cowpeas, eggplants, maize, rice, soybeans, willow, non-traditional fruits such as dragon fruit, golden berry and tree tomato as well as 41 timber species, including teak, and two forest climber species known locally as okazi and utazi.

Different kinds of organizations were also involved in the case studies. In the majority of case studies, the main organization responsible for the application of the biotechnologies was a government agency or a research institution closely linked to a governmental ministry. In some other cases, the main organization was an NGO (CS 2.2), university (CS 2.3) or a private company (2.6). In almost all cases, the main organization worked closely with a range of other institutions, including those from the private sector, UN system and/or the CGIAR, as well as with the smallholders themselves.

2. The challenges facing smallholders

The case studies looked at the application of agricultural biotechnologies to meet the challenges facing smallholders in developing countries. These challenges varied, depending on the specific place and context, but there were also many commonalities among them. A common main challenge facing farmers cited in seven of the case studies was that there was low production and that biotechnologies were applied to increase the yields, and thereby increase the farmers' income. These case studies covered production of chickpeas (CS 3.4), maize (CS 3.5), milk (CS 2.1-2.3), rice and soybeans (CS 3.2), and teak wood (CS 3.8).

For example, three case studies described how local low-producing zebu cattle in three different countries (Bangladesh, India and Malawi) were crossed with high-producing exotic dairy breeds like Holstein-Friesian or Jersey using artificial insemination (AI) to increase their milk yields (CS 2.1-2.3). For two of the case studies, an additional biotechnological approach was used with AI to face another challenge. In CS 2.2, AI was combined with semen sexing to ensure that the majority of offspring born from these matings were female (instead of an average of 50 percent) and to thereby reduce the number of unwanted male calves born in India. Male calves are a problem because many farmers simply release them. They then become a nuisance because of uncontrolled grazing and breeding, disturbances to vehicles and the spread of contagious diseases. The welfare of the calves is also compromised under these conditions. In CS 2.3, it was reported that farmers in Bangladesh indiscriminately crossbreed their cows to exotic breeds which can lead to problems of adaptability, fertility and susceptibility to heat and diseases as the offspring may have too high levels of exotic genes and be poorly adapted to the environment. The case study described how genomics was used to accurately estimate the genetic composition of crossbred cattle and to then provide farmers with advice regarding the appropriate genetic makeup of the AI bull to be used for future matings (CS 2.3).

The case studies highlighted the need for farmers to increase their yields while, equally important, facing the challenges of climate change which are now a reality. For example, the authors of CS 3.2 indicated that variety improvement for rice in Viet Nam used to emphasize productivity in the past but it currently focuses on adaptation to climate change and grain quality. With rising sea levels, salinity intrusion will affect more areas of the main rice growing regions of the country, which is a problem because of rice's sensitivity to salinity. The need to face the challenge of climate change through, *inter alia*, breeding drought tolerant crops, was therefore underlined for rice and soybean in Viet Nam (CS 3.2), chickpea in Ethiopia, India, Kenya and the United Republic of Tanzania (CS 3.4) and maize in 13 SSA countries (CS 3.5).

Five of the case studies also described how biotechnologies were used to help smallholders fight against biotic stresses. These challenges included African swine fever, a viral disease affecting domestic pigs, in Madagascar (CS 2.4); Streptococcosis, a bacterial disease affecting Nile tilapia, in Malaysia, the Philippines and Viet Nam (CS 2.6); the Mediterranean fruit fly affecting dragon fruit, golden berry and tree tomato in Ecuador (CS 3.1); the eggplant fruit and shoot borer (EFSB) affecting eggplants in Bangladesh (CS 3.3); and the pod borer affecting cowpea in Burkina Faso, Ghana and Nigeria (CS 3.5). Biotic stresses, which have become an increasing threat as a consequence of climate change, have a major impact on productivity, and thereby the farmers' incomes. The traditional approaches to treating them, such as spraying insecticides against insect pests, are expensive and can be detrimental to the environment and human health. The use of antibiotics to treat bacterial infections in fish also raises concerns about antimicrobial resistance.

Biodiversity underpins the capacity of smallholders to produce food as well as non-food products, including feed, bioenergy and bio-based materials and chemicals, in a wide range of different biophysical and socioeconomic environments. It increases resilience to shocks and stresses, provides opportunities to adapt production systems to emerging challenges and is an essential resource in efforts to increase outputs sustainably (Bélanger and Pilling, eds, 2019). It was therefore appropriate that four case studies covered aspects related to the characterization and conservation of genetic resources for food and agriculture. These included the genetic analysis of swamp buffalo populations in the Philippines in order to develop rational conservation programmes for the species (CS 2.5); the micropropagation of two forest climber species for their use in home gardens to reduce their over-exploitation in the forests in Nigeria (CS 3.6); the use of DNA tools to detect cases of illegal logging and fraudulent claims regarding timber products in India (CS 3.7); and the use of genetic analysis to identify purebred individuals of the native willow species in Argentina which is threatened by extensive hybridization with invasive exotic clones (CS 3.9).

3. The biotechnologies used as part of the solution to these challenges

As can be seen from above, the 15 case studies covered a wide range of different species, world regions and production systems. They also covered a wide range of different low- to high-tech biotechnologies. FAO traditionally uses a broad definition for biotechnology, based on Article 2 of the Convention on Biological Diversity, which states that it is "any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use". The term "agricultural biotechnologies" therefore covers a broad range of technologies used in food and agriculture. They can be applied to a range of different purposes, such as the genetic improvement of plant varieties and animal populations to increase their yields or efficiency; characterization and conservation of genetic resources for food and agriculture; and plant and animal disease diagnosis or prevention. Some of the biotechnologies can be applied to all the food and agriculture sectors, such as the use of molecular DNA markers or genetic modification, while others are more sector-specific, such as tissue culture (in crops and forest trees) or embryo transfer (livestock).

Biotechnologies vary considerably in terms of the technical, human and financial resources that they require. At the one end are biotechnologies that can be considered relatively "low-tech", which tend to be relatively simple to develop and apply as well as relatively inexpensive, with respect to the infrastructure, laboratory services or trained personnel required. These biotechnologies are well-established, have been widely used in different developing countries for a relatively long time

period and can be considered "conventional" agricultural biotechnologies. Examples include AI in livestock and micropropagation of plantlets using tissue culture, both applied in case studies here.

At the other end are a series of biotechnologies that are more "high-tech", most of which involve the direct analysis or manipulation of DNA. Examples of these, which are also featured in the case studies, include the use of DNA-based markers (such as microsatellite markers, consisting of short DNA sequences repeated many times in a given genomic location) for genetic selection; genomics-assisted breeding using thousands of single nucleotide polymorphisms (SNPs); polymerase chain reaction (PCR) based approaches for disease diagnosis and monitoring or genetic modification, where one or more genes (called transgenes) are transferred from one organism to another to introduce favourable traits.

The main biotechnologies included in these case studies involved the use of AI, DNA barcoding, doubled haploidy, genetic modification, genome-wide selection, induced mutagenesis, marker-assisted selection, micropropagation, microsatellite markers, PCR, semen sexing and the sterile insect technique. Explanations of these technical terms are provided in the case studies or can be found in Zaid *et al.* (2001) and FAO (2011). In several case studies, two or more biotechnologies were used together. For example, in Viet Nam, induced mutagenesis was used to create new rice and soybean varieties, one of which (DT80) was also produced with the use of marker-assisted selection to ensure the presence of a quantitative trait locus for salinity tolerance (CS 3.2). Similarly, CS 3.5 described how new climate-smart maize varieties were developed in SSA using a combination of doubled haploidy, marker-assisted selection and genome-wide selection.

In some cases, the same biotechnology tool was used for difference purposes. For example, microsatellite markers were used to document and investigate genetic variation among swamp buffalo populations in the Philippines (2.5); detect fraudulent claims by sellers regarding the source of teak timber in India (CS 3.7); confirm that teak plantlets produced by tissue culture in India were genetically identical to the mother plants (CS 3.8); and identify purebred individuals of an endangered willow species in Argentina (CS 3.9).

4. The obstacles to the use of biotechnologies as part of the solution to these challenges

For eight of the case studies, the authors described the challenges or obstacles they faced when applying biotechnologies. Several mentioned technical challenges, which can be quite common in scientific endeavours. For example, in developing the protocols for tissue culture of teak in India, the authors reported that problems were encountered regarding the length of time required for culture establishment, the low frequency of shoot production and the need for two-step methods for *in vitro* rooting procedures (CS 3.8). In using 30 microsatellite markers to investigate genetic diversity between animals from six swamp buffalo populations in the Philippines, the authors found that three of the markers were non-informative (not polymorphic) and so could not be used for distinguishing the populations (CS 2.5). In applying the sterile insect technology in Ecuador, the authors reported that their programme operations had technical challenges, such as the need for continuous sterile fly releases and expanding the release areas to benefit from economies of scale (CS 3.1). For the use of frozen semen in AI in Malawi, a significant challenge arose from the fact that there was just one producer of liquid nitrogen for this purpose in the country and that the irregular and interrupted supply of liquid nitrogen affected the provision of AI services to farmers.

The limited understanding by farmers of the biotechnology being applied was also cited as a challenge in some case studies, including regarding the use of microsatellite markers in buffaloes in the Philippines (CS 2.5) and the use of genetically modified (GM) eggplant in Bangladesh (CS 3.3). This was also seen as a major problem at the beginning of the project on micropropagation of two forest plants in Nigeria as many of the community members felt, incorrectly, that tissue culture would modify the plants by adding new traits (CS 3.6).

For the two case studies using genetic modification (CS 3.3 and 3.5), the authors reported some specific challenges related to the use of this technology. Regarding the development of GM eggplant varieties in Bangladesh, the challenges reported by the authors included the absence of a single regulatory entity with the necessary experience to deal with such varieties; lack of farmers' understanding of the management of GM crops, meaning that they needed a more elaborate extension programme than that which was available; and limited science-based public awareness about GM crop varieties (CS 3.3). In CS 3.5, the authors stated that the key challenges encountered in developing these new varieties included delays in regulatory approval of GM varieties as well as increased anti-biotech activism in Africa.

Other specific challenges mentioned by authors regarding the application of biotechnologies in the case studies included COVID-19, which resulted in a decrease in animal breeding activities (CS 2.2) and delays in getting results of the microsatellite analyses which had been outsourced (CS 2.5); the higher costs to the farmers of the biotechnology product (CS 2.2); the need to ensure long-term sustainability of the programme (CS 3.1) and the fact that there was only one organization involved in the commercialization or seed distribution of the new varieties (CS 3.3).

5. The impacts of applying biotechnologies as part of the solution to these challenges

The authors of all the case studies attempted in some way to describe the results that had been achieved from applying biotechnologies. The impacts reported in the case studies were consistently positive for the different indicators considered.

Economic impacts

The most common indicators of impact discussed in the case studies were of an economic nature. However, in some cases, this was not straightforward as the biotechnology products, such as new crop varieties, had only been made available to smallholder farmers recently. Furthermore, for longer living species, such as dairy cattle or teak trees, the documentation of impacts requires additional time.

For these reasons, estimates of the economic benefits in the field were not provided for some case studies but were instead the result of an *ex ante* impact assessment, used to predict the impacts that the biotechnology-driven intervention may have in the future. For example, GM pod borer-resistant cowpeas were only commercially released in Nigeria in June 2021 (CS 3.5). The case study did not discuss the impacts of the varieties released in the field but provided a summary of the positive results found in pre-release confined field trials, with respect to increased yields and reduced spraying, and reported that the results of an e*x ante* impact assessment estimated that the net present value benefits of adopting the cowpea for producers and consumers in Nigeria would

be about USD 350 million, with 70 percent accrued by producers over a period of 25 to 35 years. Case study 2.2 reported that sex-sorted semen facilities were set up at the BAIF semen station in India in November 2018 and that, based on an economic assessment considering the next five years, they predicted that the economic benefits of using sex-sorted semen vs conventional AI for dairy cattle farmers would be substantial because of the value of the additional females that are born as well as the returns from their milk.

In some other cases, even though the biotechnology products were only released recently, the authors were able to provide some early indications of their economic impacts. Nine chickpea varieties developed using genomics-assisted breeding for improved yield, drought resistance and fusarium wilt resistance were released in 2019–2022 in Ethiopia and India (CS 3.4). For two of the Indian varieties, released in 2019 and 2020, the authors estimated that, based on the demand for breeder seed, they would provide USD 19.1 million and USD 61.1 million additional economic returns respectively in the next three years. Nile tilapia that were selected for Streptococcosis resistance using genomics were released in mid-2022 in Malaysia, the Philippines and Viet Nam, for tilapia farmers from small-scale to large-scale production (CS 2.6). The authors reported that the genomically selected fingerlings represented a significant proportion of those sold in 2022 and that preliminary data suggested an average of 30 percent net profit per production cycle in the Philippines and Viet Nam.

In CS 3.8, the authors reported that large-scale production of genetically superior cloned teak began in 2016 so, obviously, the economic benefits can only be measured well into the future as the trees are normally harvested at 15–25 years of age, depending on the site conditions and management practices. They were, however, being monitored as the authors reported that initial results were encouraging as the trees exhibited fast growth in well-maintained farm fields and they suggested cloned teak can yield attractive and robust financial returns (CS 3.8).

In other cases, the case studies described long-running and well-established initiatives where the impacts of the biotechnology products were well-documented. A very good example was CS 3.2, where the authors reported that the use of induced mutagenesis for crop improvement in Viet Nam dated back to the 1970s and that by 2020, a total of 80 improved mutant crop varieties, including 54 rice and 16 soybean varieties, had been released in the country. They argued that the adoption and cultivation of induced mutant crop varieties, especially rice and soybean, had contributed to the economic development of Viet Nam. For nine rice varieties, they estimated the economic returns using data on the area of land cultivated to the variety, its increased yield (in tonnes) per ha compared to other varieties and the farmgate value of the produce per tonne for the farmer. For example, they estimated that the rice mutant variety DT 10 had been cultivated on a cumulative area of 2.4 million ha between 1990 and 2014, benefiting 3 million farmers and increasing their income by USD 450 million. Similarly for four soybean varieties developed using induced mutagenesis, they estimated that in 2000–2019 they were cultivated on a cumulative area of 156 000 hectares, giving an estimated economic benefit of USD 58.6 million.

The authors of the case study on GM eggplant also provided good documentation of the economic impacts at the farmer level (CS 3.3). The first varieties were approved in 2013 and scaling up began in 2019. The authors reported that farmers who planted GM eggplants saved 61 percent of pesticide costs and earned a net return of USD 2151/ha compared to USD 357/ha for non-GM eggplants. They also reported that an estimated 18 percent of the winter eggplant area was under GM eggplant cultivation in the 2020/21 growing season.

Another good example was the cultivation of non-GM drought resistant maize on a cumulative area of about 1 million ha from 2013 to 2020 in 13 SSA countries (Benin, Cameroon, Ethiopia, Ghana, Kenya, Mozambique, Nigeria, Rwanda, South Africa, the United Republic of Tanzania, Uganda, Zambia and Zimbabwe) (CS 3.5). The authors reported that an external impact evaluation in three countries in 2021 showed that the productivity of maize and the consequent income generation for farmers increased significantly with the adoption of the new varieties.

In other cases, while comprehensive impact assessment studies were not reported, the economic benefits of applying the biotechnologies were still evident. Programmes to reduce fruit fly prevalence, involving use of the sterile insect technique, began in Ecuador in 2014 (CS 3.1). As a result of maintaining fly populations at low prevalence levels in fruit plantations in Ecuador, they reported that the export of dragon fruit, tree tomato and golden berry from Ecuador to the United States of America was approved for the first time in 2017, 2018 and 2019 respectively and that negotiations to export to a number of other countries were also initiated. For the benefits of using AI in Malawi, the authors reported that a detailed economic analysis of the operations of a typical experienced farmer who applied AI, using real farm data, estimated that the farmer had net earnings of almost USD 2 per milking cow per day from milk sales.

Furthermore, two of the case studies mentioned the impacts on rural employment which, obviously, had indirect economic benefits for the community as a whole. Case study 2.1 described how the use of AI for dairy cattle production in Malawi, and the growth of the national dairy industry, provided employment for people in the support services along the dairy value chain, including people working as AI technicians and selling grass to farmers (CS 2.1). Case study 3.6 described how community members in Nigeria grew two tissue culture-derived forest climbers in their home gardens and began to sell vegetables of these species within their local communities and to access new markets in cities of the state.

Non-economic impacts

While economic benefits to the farmers are fundamental, many case studies also documented how application of biotechnologies had other kinds of impacts that did not have any direct economic benefit (although they might have indirect economic benefits immediately or sometime in the future).

Among these were the use of biotechnology tools to detect African swine fever (CS 2.4), a highly contagious viral disease for which there is currently no effective treatment or vaccine. The authors reported that they used PCR for early detection of African swine fever in seven suspected outbreaks of the disease in Madagascar in 2019–2022. Pigs on the farms where positive animals were detected were slaughtered, which prevented further transmission of the disease (CS 2.4). Another involved the use of genomics information to determine the local/exotic genetic composition of crossbred dairy cows and, based on this, to advise farmers on the appropriate genetic make-up of the sire to be used for mating with AI (CS 2.3). This advice is very useful because, in general, the productivity of crossbred cattle increases with rising levels of exotic genes, but they need to be supported by the appropriate feeding, housing and veterinary environment, and so the matching of the genetic make-up to the farmers' conditions is important.

A number of case studies showed how the application of biotechnologies had reduced the use of pesticides. This, apart from the economic benefits, also had potential positive benefits on the environment and human health. Through the use of the sterile insect technique as part of an integrated pest management approach to control fruit flies in Ecuador, the authors reported that the

fly populations were brought down to low prevalence levels without the need for insecticides to be sprayed in the environment (CS 3.1). Through the use of pest resistant GM eggplants in Bangladesh, the authors reported that farmers applied fewer pesticides and spent 61 percent less on pesticides compared to non-GM eggplant farmers (CS 3.3). For pest resistant GM cowpeas, released in Nigeria in 2021 and currently in the pipeline for approval in Burkina Faso and Ghana, data on post-release impacts were not provided (CS 3.5). Nevertheless, the authors reported that confined field trials of the new varieties in these countries showed that the frequency of the spraying of insecticides was reduced from six to ten times per season to just two, representing a saving of four litres of insecticide per ha or USD 12.5 per ha.

The case studies also showed that biotechnologies can have a positive impact on the characterization and conservation of genetic resources for food and agriculture. The authors of CS 2.5 reported that the findings of their analysis of the genetic diversity among swamp buffalo populations in the Philippines had provided important insights, leading to clear recommendations regarding how to best conserve the species in the country. Case study 3.6 described the micropropagation of two forest climber species in Nigeria and their distribution to community members for cultivation in home gardens. They reported that the project, funded by the Global Environment Facility in 2009–2010, successfully increased the availability of the plants in the community and reduced their exploitation in the forest and that at the end of the project, there were more new climbers of the species in the forest compared to the start of the project. The author also reported that recent information from the community (in 2022) indicated that there was increased regeneration of the two species in the forest (CS 3.6).

In Argentina, microsatellite marker analysis showed that the native willow *Salix humboldtiana* was under serious threat because of its hybridization with highly vigorous and invasive exotic *Salix* clones (CS 3.9). Based on the results of the genetic analysis, a participatory rescue programme was set up to conserve the species where raising public awareness was one of its key activities. They reported that microsatellite markers results were very important in motivating people to conserve the species.

In India, because of the huge demand for wood and inadequate domestic supply, illegal felling of trees and false claims of timber origin are a problem where commercially traded timber species are often adulterated with timber from an inferior quality species (CS 3.7). The authors reported that DNA barcodes, i.e. short stretches of species-specific gene sequences, were used to identify cases of misrepresentation and fraudulent claims made by the wood suppliers. In addition, for teak, which is a highly valued tropical timber species, microsatellite markers had been used to develop a database making it possible to identify teak timber from different parts of India. They reported that this database had been used in court cases related to the illegal felling of timber and that it had helped the Kerala State Forest Department to produce scientific evidence and take legal actions against the culprits (CS 3.7).

6. The key ingredients needed for success

Although each case study had its specificities related to its context and place, from looking collectively at the case studies, four main ingredients could be identified for the successful application of agricultural biotechnologies to meet the needs of smallholders in developing countries.

Partnerships

The first, and arguably the most important, ingredient for success identified from these case studies was close partnerships between researchers and relevant institutions in developing countries with those in advanced research institutions, CGIAR centres, the private sector and/ or UN bodies. In these case studies, researchers and institutions in developing countries worked closely with partners all over the world.

These included research institutions and universities in Organisation for Economic Co-operation and Development (OECD) countries, including Cornell University in the United States of America (CS 3.3), the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Australia and the Donald Danforth Plant Science Center and Purdue University in the United States of America (CS 3.5) and the University of Marburg in Germany (CS 3.9). These included CGIAR centres, namely the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (CS 3.4) and the International Maize and Wheat Improvement Center (CIMMYT) and the International Food Policy Research Institute (IFPRI) (CS 3.5).

These also included entities that are part of the UN system, such as the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture (CS 2.3, 3.1 and 3.2), the World Bank, that provided funding for activities in CS 3.8, and the Global Environment Facility that, through an initiative implemented by the United Nations Development Programme, provided funding for activities in CS 3.6.

The partnerships described in some of the case studies (especially CS 3.3, 3.4 and 3.5) were complex, involving many partners and countries. For the public-private partnership (PPP) established to develop new varieties of climate-smart maize, the partners included Bayer Crop Science, CIMMYT and the national agricultural research systems (NARS) of Ethiopia, Kenya, Mozambique, Nigeria, South Africa, Uganda and the United Republic of Tanzania (CS 3.5). For the development of pod borer resistant cowpea varieties, the cooperation involved Bayer Crop Science, CSIRO, the Donald Danforth Plant Science Center, IFPRI, Purdue University and the NARS of Burkina Faso, Ghana and Nigeria. The African Agricultural Technology Foundation (AATF) coordinated both of these PPPs (CS 3.5).

As noted by the authors in CS 3.3, partnerships made it possible to enable access to proprietary technologies and the provision of long-term funding. The case studies provided some good examples where PPPs enabled the researchers and institutions in developing countries to gain access to proprietary materials or technologies. These included the sexing semen technology developed by the company Sexing Technologies, for its use in India (CS 2.2); the pest resistant gene from Mahyco for use in eggplants in Bangladesh (CS 3.3); and the genes for pest resistance and drought tolerance in maize and for pest resistance in cowpea from Bayer Crop Science for use in SSA (CS 3.5).

The establishment of solid, broad partnerships almost certainly played a central role in securing funding for many of the initiatives described in these case studies, such as those that received funding from the United States Department of Agriculture (CS 2.4); the United States Agency for International Development (USAID) (CS 3.3); the Bill & Melinda Gates Foundation (BMGF), CGIAR Generation Challenge Program and the Government of India (CS 3.4); BMGF, USAID and the Howard G. Buffett Foundation (maize, CS 3.5); and BMGF, USAID and the Rockefeller Foundation (cowpea, CS 3.5).

As noted by the authors in CS 3.1, partnerships – in their case between the relevant government agency (Agrocalidad), IAEA, FAO and the fruit growers – allowed for the concerted investment of efforts and the pooling of resources. This was also seen in CS 2.3 where the researchers in Bangladesh were able to benefit from the state-of-the-art DNA microarray technology used at the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture to establish the genetic make-up of their crossbred cattle.

For complex multistakeholder and/or multicountry partnerships, it is essential that each of the bodies has clearly defined and complementary roles. The detailed description of the roles of the different partners in the PPPs developed for eggplant in CS 3.3 and for both maize and cowpea in CS 3.5 illustrated this nicely.

Investments in agricultural research worldwide are extremely unbalanced. A small number of high- and middle-income countries are responsible for the majority of the investments while, at the other extreme, spending by low-income countries is very low. In this situation, researchers and their institutions in developing countries need to actively forge international partnerships, such as those described here, to seek to address the needs of their smallholders (Ruane and Ramasamy, 2023).

Long-term commitment

The second ingredient for success was long-term sustained commitment, both financial and non-financial, from stakeholders in the initiative.

This was well exemplified by the case study of GM eggplant in Bangladesh (CS 3.3). In 2003, the technology was sublicensed by the Indian company Mahyco to the public sector Bangladesh Agricultural Research Institute. The first varieties were approved in 2013 and the seeds were supplied to 20 farmers for cultivation in 2014. Scaling up began in 2019. USAID has provided long-term stable funding to this work through three different projects, namely the Agricultural Biotechnology Support Project II, awarded in 2002, which was followed by the Feed the Future South Asia Eggplant Partnership and then the current Feed the Future Insect-Resistant Eggplant Partnership. The Government of Bangladesh, through the work of three of its institutes covering research, extension and seed production/distribution, has also shown similar commitment over this long time period (CS 3.3).

After many years of research, the draft genome sequence of chickpea was published in a scientific journal in 2013 (CS 3.4). Building on this and other genomics resources, the case study reported that nine new varieties developed using genomics-assisted breeding were released in 2019–2022 in Ethiopia and India for yield, drought resistance and fusarium wilt resistance and that high yielding and drought resistance lines were developed and ready for possible release in Kenya and the United Republic of Tanzania in 2023. For the use of the sterile insect technology to control the

fruit fly in Ecuador, the authors underlined that long-term commitment, coordination and active participation of the different stakeholders had been important for the success of their initiative (CS 3.1).

In maize, the programme to develop climate-smart varieties, both GM and non-GM, began in 2008 in seven countries (CS 3.5). While release of the non-GM varieties had been relatively quick, beginning in 2013, progress with GM varieties had been slower. GM maize was released in South Africa while it was still in the pipeline in Ethiopia, Kenya, Mozambique and Nigeria. For GM cowpea, field trials took place from 2011 to 2016 in Burkina Faso, Ghana, Nigeria. They were released in Nigeria in 2021 and were still in the pipeline in the two other countries (CS 3.5).

While the long time period between the initiation of programme activities and the delivery of biotechnology products to smallholders in these examples shows that long-term and stable commitment is essential for successful application of biotechnologies in crops, it is even more so in species with longer life cycles. For example, CS 3.8 reported the results of field trials of genetically improved cloned teak which lasted 18 years.

The point about the importance of long-term commitment, which emerged from these case studies, was also underlined by Alston, Pardey and Rao (2021) who wrote: "Agricultural research is slow magic. Returns accrue over long periods – decades – and realizing the full potential from agricultural R&D requires far-sighted investments. It is also a cumulative endeavour, best done with steady and sustained investments. The accumulated evidence shows that, in agricultural R&D, persistence and patience are well rewarded."

Government support

The third key ingredient for success identified from the case studies was political and financial commitment from national and/or local governments.

This was evident in CS 3.3 where the authors underlined the role of the government in success of the initiative, indicating that political will by the government created the enabling environment, for both R&D activities to be conducted and the outputs to be disseminated, and that there was an identified constraint (eggplant productivity damaged by EFSB infestations) for which a solution had to be found, and the government led the way.

Similar determination by the government to support the search for a solution to an important constraint was evident in CS 3.1 where the Government of Ecuador provided investment funds for six years to the National Fruit Fly Project, aiming to control the Mediterranean fruit fly by establishing and maintaining fruit fly-free and low prevalence areas in the country, which started in 2014. The authors also reported that the government had recently launched a new follow-up National Project, to continue developing fruit fly low prevalence and free areas.

Case Study 3.8 indicated the central role of the government in launching a national programme in 2019–20 on teak cloning in India to popularize small-scale clonal plantations across the country. The support of state governments was also emphasized, where the authors reported that the main success factors for the initiative were that the forest departments of teak-growing states in India, such as the Forest Development Corporation of Maharashtra State and the Kerala Forest Department, supported the project and involved various stakeholders such as farmer associations, NGOs, the private sector and academia (CS 3.8). The importance of national and local government

support was also underlined in CS 2.5 where the authors reported that the initiative to document swamp buffalo genetic diversity in the Philippines was funded by the Department of Agriculture and that the local governments supported the establishment of swamp buffalo sanctuaries in Calayan Island and Pitogo Island.

The use of sex-sorted semen offered substantial potential economic and societal benefits but the major hurdle to its use was the cost, which was almost six times higher than conventional AI (CS 2.2). Subsidies to reduce the cost were therefore fundamental for its adoption by smallholder farmers. In addition to dairy cooperatives and private companies, the authors reported that subsidies to reduce the costs of sex-sorted semen were provided by state governments, such as Uttar Pradesh, Jharkhand and Odisha and government agencies.

Good communication

The importance of good communication with farmers and other relevant stakeholders was highlighted in the case studies as a key ingredient of success.

In case study 2.2, the authors described the extensive efforts they put into communication with smallholder farmers, including through village-level farmer meetings, posters, local newspaper articles, social media and call centres, and said they were one of the factors behind the programme's success. Furthermore, one of the challenges seen with the use of sex-sorted semen was that an average of 90 percent of offspring were female but 10 percent were male, which obviously would be a disappointment for the farmers who paid for these inseminations. To ensure they were aware of this and to prevent potential conflicts once the offspring were born, inseminations were only carried out after getting a signed consent form from the farmer which informed them about the 10 percent possibility of producing a male calf (CS 2.2).

Rather than controlling the fruit flies on an orchard-by-orchard basis, the sterile insect technique was applied on an area-wide basis, covering the total pest population in an area, as part of an integrated pest management approach (CS 3.1). To ensure the active involvement and coordination of all relevant stakeholders, they noted that the dissemination of information and adequate communication to the fruit growers and the general public regarding the activities of the programme, as well as the benefits obtained, were essential for its success.

For the case study of pest resistant GM eggplant in Bangladesh, the authors underlined the importance of communication (CS 3.3). They noted that significant efforts were invested in involving farmers in the trialing of new varieties, which they said made them co-creators of the solution and therefore enhanced the adoption of the new varieties. In addition, they noted that several farmer field days were conducted by the Bangladesh Agricultural Research Institute in different districts to demonstrate the improved traits of the new varieties and their production practices to sensitize the farmers to the benefits of GM eggplant. They reported that the creation of public awareness regarding the safety and utility of the GM eggplants in the country was also critical (CS 3.3).

At the beginning of the project to use tissue culture for micropropagation of two forest plants in Nigeria, there were misplaced fears by many community members that tissue culture would result in modification of the plants to add new traits (CS 3.6). To mitigate this, the author reported that the project developed a communication strategy to build trust among community members and that the use of focus groups and follow-up discussion methods, as well as an emphasis on

local ownership so the community was made to own the project, were key to the tissue culture plantlets being accepted by the community.

In CS 3.9, the author described the numerous activities carried out over their 12-year programme to rescue the endangered native willow species in Argentina, including an extensive awareness raising campaign involving, *inter alia*, meetings with Patagonian community authorities and many talks in rural schools. The author reported that his research institute had lengthy experience of working with rural people and local communities in similar projects and that presentation of the microsatellite markers results was important for public awareness and helped to motivate people to conserve the species.

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In 2022, FAO made a worldwide call inviting stakeholders to submit abstracts of case studies where agricultural biotechnologies had been applied to solve the problems of smallholders in developing countries in the aquaculture, crop, forestry and livestock sectors. The 15 case studies selected here cover a wide range of different species, world regions and production systems. They also discuss a variety of low- to high-tech biotechnologies.

The case studies describe how biotechnologies were applied in different situations to increase the smallholders' yields in the face of climate change, and thereby increase smallholder incomes as well as helping them fight against biotic stresses. They also describe how biotechnologies were used for the characterization and conservation of genetic resources for food and agriculture.

From a collective consideration of all case studies, four key ingredients for the successful application of biotechnologies to meet the needs of smallholders in developing countries were identified. The first, and arguably the most important, were close partnerships between researchers and relevant institutions in developing countries with those in advanced research institutions, CGIAR centres, the private sector and/or UN bodies. The second was long-term sustained commitment, financial and non-financial, from stakeholders in the initiative. The third was political and/or financial support from national and/or local governments. The fourth was good communication with farmers and other relevant stakeholders.