

## Learning from the past: Successes and failures with agricultural biotechnologies in developing countries over the last 20 years

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#### 1. Introduction

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) will take place in Guadalajara, Mexico in early 2010. ABDC-10 is co-organized by FAO and the Government of Mexico (<http://www.fao.org/biotech/abdc/conference-home/en/>).

Impetus for the conference comes from the need for concrete steps to be taken to move beyond the “business-as-usual” approach and to respond to the growing food insecurity in developing countries, particularly in light of climate change that will worsen the living conditions of farmers, fishers and forest-dependent people who are already vulnerable and food insecure. The recent increases in food prices have had dramatic consequences globally. In October 2008, FAO released its major report on “The State of Food Insecurity in the World” indicating that in 2007, mainly because of rising food prices, the number of hungry people in the world increased by 75 million (FAO, 2008a). Although international prices have now declined somewhat, the problems of food insecurity and hunger remain and the challenges they pose are particularly difficult for the rural poor, who make up an estimated 75 percent of the world's 963 million hungry people.

ABDC-10 aims to be a stock-taking exercise across the different food and agricultural sectors, describing the current status and analysing previous successes/failures in order to learn from the past and make recommendations for the future. The ability to look back and learn from the past is possible

because a large number of biotechnology tools are available and some of them have already been used for many years in a wide range of developing countries. For example, a survey carried out by FAO nearly 20 years ago on the use of artificial insemination indicated that over 16 million cattle were inseminated in developing countries in 1990/1991 (Chupin, 1992). One of the expected outputs from ABDC-10 is therefore an analysis of the reasons for the success and failure of application of different biotechnologies in developing countries in the past (<http://www.fao.org/biotech/abdc/about/confoutputs/en/>).

As part of the build up to ABDC-10, FAO is preparing a series of five technical sector-specific documents, on biotechnology applications in crops, forestry, livestock, fisheries and aquaculture and, finally, food processing and food safety (FAO, 2009a-e). Each one aims to document the current status of application of biotechnologies in developing countries in its sector; provide an analysis of the reasons for successes/failures of application of biotechnologies in developing countries; present some relevant case studies; and make recommendations for the future (<http://www.fao.org/biotech/abdc/backdocs/en/>). To complement these documents, the FAO Biotechnology Forum is hosting this cross-sectoral e-mail conference on “Learning from the past: Successes and failures with agricultural biotechnologies in developing countries over the last 20 years” to bring together and discuss relevant, often un-documented, past experiences of applying biotechnologies in developing countries, ascertain the success or failure (partial or full) of these experiences, and determine and evaluate the key factors that were responsible for their success or failure.

In this e-mail conference, as well as ABDC-10, the term agricultural biotechnology encompasses a variety of technologies used in food and agriculture, for a range of different purposes such as the genetic improvement of plant varieties and animal populations to increase their yields or efficiency; genetic characterization and conservation of genetic resources; plant or animal disease diagnosis; vaccine development; and improvement of feeds. Some of these technologies may be applied to all the food and agricultural sectors, such as the use of molecular markers or genetic modification, while others are more sector-specific, such as tissue culture (in crops and forest trees), embryo transfer (livestock) or sex-reversal (fish). Note, the term agriculture includes the production of crops, livestock, fish and forestry products, so the term ‘agricultural biotechnologies’ encompasses their use in any of these sectors.

This Background Document aims to provide information that participants will find useful for the e-mail conference. In Section 2 an overview is provided of the different agricultural biotechnologies to be considered. Section 3 presents some specific guidance about this e-mail conference. Section 4 provides references of articles mentioned in the document, abbreviations and acknowledgements.

## 2. Agricultural Biotechnologies in Developing Countries

Here we provide a brief overview of the main kinds of agricultural biotechnologies that have been used in developing countries over the past 20 years and that should be covered in the e-mail conference. They are described separately, although in practice more than one may be used together in certain situations (e.g. in wide crossing programs, see Section 2.8.3). Note, new biotechnologies that are still at the research level, be it in the laboratory or at the field trial stage, but which have not yet been applied (i.e. used for commercial production by farmers) in developing countries are not included.

A short description of the different biotechnologies is provided below, indicating also what they are used for, the food and agricultural sectors involved and giving some examples of their applications in specific developing countries. Regarding the examples, their inclusion in the document does not imply that these applications have been a partial or complete success (or, conversely, that they have been any kind of a failure). Indeed, these are the kind of issues to be addressed by participants during this e-mail conference. Also, it should be kept in mind that, although not the subject of this e-mail conference, the pathway from a research development in the laboratory to its eventual application in the field (e.g.

farmers cultivating a new genetically improved plant variety or using a new vaccine against an animal disease) can be long, resource-demanding and unsuccessful, so many biotechnologies of seemingly high promise at the experimental stage have had limited applications in developing countries so far.

As many of the biotechnologies described below are related to molecular biology and genetic material, some basic terminology is introduced here. Living things are made up of cells that are programmed by genetic material called DNA. A DNA molecule is made up of a long chain of nitrogen-containing bases. Only a small fraction of this DNA sequence typically makes up genes i.e. that code for proteins, which are molecules essential for the functioning of living cells, made up of chains of amino acids. The remaining and major share of the DNA represents non-coding sequences whose role is not yet clearly understood. The genetic material is organized into sets of chromosomes (e.g. 5 pairs in *Arabidopsis thaliana* – a model plant species; 30 pairs in cattle), and the entire set is called the genome. In a diploid individual (i.e. where chromosomes are organized in pairs), there are two alleles of every gene - one from each parent – transmitted by gametes (reproductive cells) that are normally haploid (having just one of each of the pairs of chromosomes). A typical genome contains several thousand genes e.g. about 30,000 genes in grasses like rice and sorghum (Paterson et al, 2009). Definitions of technical terms used below can be found in the FAO Biotechnology Glossary ([http://www.fao.org/biotech/index\\_glossary.asp](http://www.fao.org/biotech/index_glossary.asp)).

## 2.1 Molecular markers

Molecular markers are identifiable DNA sequences, found at specific locations of the genome, transmitted by standard Mendelian laws of inheritance from one generation to the next. They rely on a DNA assay and a range of different kinds of molecular marker systems exist, such as restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs) and microsatellites. The technology has improved in the past decade and faster, cheaper systems like single nucleotide polymorphisms (SNPs) are increasingly being used. The different marker systems may vary in aspects such as their technical requirements, the amount of time, money and labour needed and the number of genetic markers that can be detected throughout the genome.

Molecular markers have been used in laboratories since the late 1970s and they are applied across all the food and agricultural sectors. They are very versatile and can be used for a variety of purposes. Thus, they are used in genetic improvement, through so-called marker-assisted selection (MAS), where markers physically located beside (or, even, within) genes of interest (such as those affecting yield in maize) are used to select favourable variants of the genes (FAO, 2007a). MAS is made possible by the development of molecular marker maps, where many markers of known location are interspersed at relatively short intervals throughout the genome, and the subsequent testing for statistical associations between marker variants and the traits of interest. Marker maps are now available for a wide range of economically important agricultural species (see e.g. FAO, 2007a for details). Progress in the field of genomics (the study of an organism's entire genome) has also provided much useful information for MAS, enabling in some cases markers to be used that are located within the genes of interest.

Molecular markers are also used to characterize and conserve genetic resources, where some of the approaches can be applied in each of the crop, forestry, livestock and fishery sectors (e.g. estimating the genetic relationships between populations within a species). Other uses again are more sector-specific, such as their utilization to identify duplicate accessions in crop genebanks; monitor effective population sizes ( $N_e$ ) in capture fish populations or carry out biological studies (e.g. of mating systems, pollen movement and seed dispersal) in forest tree populations (Ruane and Sonnino, 2006a). They are also used in disease diagnosis, to characterize and detect pathogens in livestock, crops, forest trees, fish and food (see Section 2.4).

Molecular markers have been used in a number of developing countries. In livestock, for example, they have been used in four African countries for characterization of genetic resources and in eight

Asian countries, where six used them for genetic distance studies and two for MAS (FAO, 2007b). In Latin America and the Caribbean, most countries have used molecular techniques, primarily for characterization purposes, while their use has been limited in the Near and Middle East (FAO, 2007b). In crops, several examples of new hybrids and varieties developed through MAS are available, and in progress, in different crops, such as pearl millet, rice and maize, and in several developing countries like Bangladesh, India and Thailand (Varshney et al, 2006). Different centres of the Consultative Group on International Agricultural Research (CGIAR) have been working with partners in developing countries to accelerate plant breeding practices through MAS.

## 2.2 Genetic modification

A genetically modified organism (GMO) is an organism in which one or more genes (called transgenes) have been introduced into its genetic material from another organism. The genes may be from a different kingdom (e.g. a bacterial gene introduced into plant genetic material), a different species within the same kingdom or even from the same species. For example, so-called 'Bt crops' are crops containing genes derived from the soil bacterium *Bacillus thuringiensis* coding for proteins that are toxic to insect pests that feed on the crops. The issue of GMOs has been highly controversial over the past decade. Many countries have introduced specific frameworks to regulate their release and commercialization.

GM crops were first grown commercially in the mid 1990s. While the majority continues to be grown in developed countries, an increasing number of developing countries are reported to be cultivating them. Recent estimates (James, 2008) indicate that 10 developing countries planted over 50,000 hectares of GM crops in 2008 i.e. Argentina (21.0 million hectares), Brazil (15.8), India (7.6), China (3.8), Paraguay (2.7), South Africa (1.8), Uruguay (0.7), Bolivia (0.6), Philippines (0.4) and Mexico (0.1). For comparison, in 1997 the only developing countries reported were Argentina (1.4 million hectares), China (1.8) and Mexico (less than 0.1). Almost all GM crops grown commercially are genetically modified for one or both of two main traits: herbicide tolerance (63% of GM crops planted in 2008) or insect resistance (15%), i.e. Bt crops, while 22% have both traits (James, 2008).

Commercial release of GM forest trees has been reported in one country, China. In 2002, approval was granted for the environmental release of two kinds of Bt trees, the European black poplar (*Populus nigra*) and the hybrid white poplar clone GM 741, together representing about 1.4 million plants on 300-500 hectares (FAO, 2004). Regarding GM livestock or fish, there has been no commercial release for food and agriculture purposes in any developing or developed country.

Although documentation is generally quite poor, use of genetically modified micro-organisms (GMMs) in the agro-industry and animal feed sector is routine in developed countries and is also a reality in many developing countries. In the agro-industry sector, use of enzymes, i.e. proteins that catalyse specific chemical reactions, is important. Many of the enzymes used in the food industry are commonly produced using GMMs. For example, since the early 1990s, preparations containing chymosin (an enzyme used to curdle milk in the preliminary steps of cheese manufacture) derived from GM bacteria have been available commercially (Ruane and Sonnino, 2006b). Similarly, many colours, vitamins and essential amino acids used in the food industry are also from GMMs.

In animal nutrition, feed additives such as amino acids and enzymes are widely used in developing countries. The greatest use is in pig and poultry production where, over the last decade, intensification has increased, further accelerating the demand for feed additives. For example, most grain-based livestock feeds are deficient in essential amino acids such as lysine, methionine and tryptophan and for high producing monogastric animals (pigs and poultry) these amino acids are added to diets to increase productivity. The amino acids in feed, L-lysine, DL-methionine, L-threonine and L-tryptophan, constitute over half of the total amino acid market. In India alone, the amino acid market amounts to about 5 million US dollars. The essential amino acids are produced in some cases by GM strains of *Escherichia coli* (FAO, 2009c).

In the dairy industry, recombinant bovine somatotropin (rBST), a protein hormone from an *Escherichia coli* K-12 bacterium containing the cow somatotropin gene, has been used to increase milk production in a number of developing countries. Chauvet and Ochoa (1996) report that rBST was first used in Mexico in 1990 and has been sold in a number of other developing countries, including Brazil, Malaysia, South Africa and Zimbabwe.

### 2.3 Chromosome number manipulation

As mentioned earlier, genetic material is organized into sets of chromosomes and each plant and animal species has a characteristic number of chromosomes. Manipulation of whole sets of chromosomes is possible and is used for a range of different purposes in agriculture. For example, fish and shellfish have been extensively studied for manipulation of their chromosomes during early stages of development. Using relatively simple techniques such as cold and hot shocks it is possible to induce triploidy (i.e. with three sets of chromosomes), leading to the production of nearly completely sterile populations. Sterility may be desirable in conservation programs, where it can prevent introgression of escaped individuals from commercial stocks into natural populations. It may also be of interest in commercial fish operations, e.g. when developing hybrid stocks or to prevent the side effects of sexual maturation on carcass quality (FAO, 2009d). As in fish, induction of sterility in crops may be desirable in certain breeding programmes, e.g. to produce seedless fruits, and one of the most rapid and cost-effective approaches is to create polyploids (i.e. with more than 2 complete sets of chromosomes), especially triploids. Triploid varieties have been produced in numerous fruit crops including most of the citrus fruits, acacias and the kiwifruit (FAO, 2009a).

Another example of chromosomal number modification in fish is the production of haploid individuals after eggs are fertilized by sperm which do not contribute genetic material (a process called gynogenesis) or when normal sperm fertilize eggs whose DNA has been deactivated (a process called androgenesis). In both cases the haploid chromosomes can then be duplicated using shocks. The importance of gynogenesis/androgenesis is that it is possible to develop inbred individuals, which may be useful in fish breeding experiments aimed at producing clonal lines for detecting genomic regions affecting quantitative traits (FAO, 2009d).

In crops, chromosome doubling is one of the most important technologies for the creation of fertile inter-specific hybrids (wide-crosses). Wide crossing involves hybridizing a crop variety with a distantly related plant from outside its normal sexually compatible gene pool. Its usual purpose is to obtain a plant that is virtually identical to the original crop, except for a few genes contributed by the distant relative. The technique has enabled breeders to access genetic variation beyond the normal reproductive barriers of their crops (FAO, 2009a). For example, the New Rice for Africa (NERICA) hybrids are derived from crossing two species of cultivated rice, the African rice and the Asian rice, combining the high yields from the Asian rice with the ability of the African rice to thrive in harsh environments.

Wide-hybrid plants are often sterile so their seed cannot be propagated, due to differences between the sets of chromosomes inherited from genetically divergent parental species that prevent stable chromosome pairing during meiosis. However, if chromosome number is artificially doubled, the hybrid may be able to produce functional pollen and eggs and be fertile. Colchicine has been used for chromosome doubling in plants since the 1940s and has been applied to more than 50 plant species, including most important annual crops. More recently, several additional chromosome doubling agents, all of which act as inhibitors of mitotic cell division, have been used in plant breeding programmes. To date, with the help of chromosome doubling technology, hundreds of new varieties have been produced worldwide (FAO, 2009a).

In crops and forest trees, chromosome doubling has also been used, as for fish, to generate 'doubled haploids'. The haploid plants can be produced using anther culture which involves the *in vitro* culture of immature anthers (i.e. the pollen-producing organs of the plant). As the pollen grains are haploid, the resulting pollen-derived plants are also haploid (Sonnino et al, 2009). Doubled haploid plants were

first produced in the 1960s using colchicine and today, thermal shock or mannitol incubation can be used. They may also be produced from ovule culture. Breeders value doubled haploid plants because they are 100% homozygous so any recessive genes are readily apparent. The time required after a conventional hybridization to select pure lines carrying the required recombination of characters is thus drastically reduced. Since the 1970s, doubled haploid methods have been used to create new varieties of barley, wheat, rice, melon, pepper, tobacco, and several Brassicas. In the developing world, a major centre of such breeding work is China, where numerous haploid crops have been released and many more are being developed. By 2003, China was cultivating over 2 million hectares of doubled haploid varieties, the most important of which are rice, wheat, tobacco and peppers (FAO, 2009a).

## 2.4 Biotechnology-based diagnostics

Applications of biotechnology for diagnostic purposes are important in crops, forest trees, livestock and fish as well as for food safety purposes. Two main kinds of methods are used, those based on the enzyme-linked immunosorbent assay (ELISA) and those based on the polymerase chain reaction (PCR).

ELISA systems are antibody-based techniques for the determination of the presence and quantity of specific molecules in a mixed sample. They are used in a range of formats, both for the detection of pathogens and for detection of antibodies produced by the host as a response to the pathogens, and a range of commercial kits are available, e.g. to detect fish and shrimp pathogens (Adams and Thompson, 2008). Some of the ELISA-based methods use monoclonal antibodies, produced by a cell line that is both immortal and able to produce highly specific antibodies, or polyclonal antibodies, produced by many cell lines. In livestock, ELISAs form the large majority of prescribed tests for the OIE notifiable animal diseases, and many diagnostic kits are available in developing countries (FAO, 2009c).

The PCR-based methods rely on the fact that each species of pathogen carries a unique DNA or RNA sequence that can be used to identify it. PCR allows production of a large quantity of a desired DNA from a complex mixture of heterogeneous sequences. It can amplify a selected region of 50 to several thousand DNA base pairs into billions of copies. After amplification, the target DNA can be identified using techniques such as gel electrophoresis or hybridization with a labeled nucleic acid (a probe). Real time PCR (or quantitative PCR) enables quantification of DNA or RNA present in a sample. The genomes of some viruses, such as the influenza A virus, are made of RNA instead of DNA, and to identify RNA from these viruses a complementary DNA (cDNA) copy of the RNA is first synthesized using an enzyme called reverse transcriptase. The cDNA then acts as the template to be amplified by PCR (FAO, 2009c). This method is called reverse transcriptase PCR (RT-PCR).

PCR-based techniques offer high sensitivity and specificity and diagnostic kits allow the rapid screening of the virus or bacteria and have a direct use in situations where individuals show no antibody response after infection. For example, molluscs do not produce antibodies, and therefore antibody-based diagnostic tests are limited in their application to pathogen detection in these species. In fisheries, PCR-related tools are increasingly being used in developing countries, although they require detailed knowledge of the genomics of the pathogen itself and extensive validation in practice (FAO, 2009d).

In livestock, public sector production of diagnostic kits for animal diseases in Asia and Latin America can be found in Brazil, Chile, China, India, Mexico and Thailand. Research capabilities for development, standardization and validation of diagnostic methods are also well advanced in these countries. PCR-based diagnostics are increasingly being employed in developing countries to back up findings from serological analyses. However, their use is largely restricted to laboratories of research institutions and universities and to the central and regional diagnostic laboratories run by governments (FAO, 2009c). In aquaculture, there are some highly integrated companies operating in developing countries (e.g. in shrimp production) and these companies commonly use PCR-based diagnostic

systems, where the analyses are either carried out by laboratories of the companies themselves or are outsourced to specialized private laboratories.

Biotechnology-based diagnostics are also important in food analysis. Many of the classical food microbiological methods used in the past were culture-based, with micro-organisms grown on agar plates and detected through biochemical identification. These methods are often tedious, labour-intensive and slow. Genetic based diagnostic and identification systems can greatly enhance the specificity, sensitivity and speed of microbial testing. Molecular typing methodologies, commonly involving PCR, ribotyping (a method to determine homologies and differences between bacteria at the species or subspecies/strain level, using RFLP analysis of ribosomal RNA genes) and pulsed-field gel electrophoresis (a method of separating large DNA molecules on agarose gels), can be used to characterize and monitor the presence of spoilage flora (microbes causing food to become unfit for eating), normal flora and microflora in foods (Ruane and Sonnino, 2006b, chap. 6.1). RAPD or AFLP molecular marker systems can also be used for the comparison of genetic differences among species, subspecies and strains, depending on the reaction conditions used. The use of combinations of these technologies and other genetic tests allows the characterization and identification of organisms at the genus, species, subspecies and even strain levels, thereby making it possible to pinpoint sources of food contamination, to trace micro-organisms throughout the food chain or to identify the causal agents of food-borne illnesses (Ruane and Sonnino, 2006b).

## 2.5 Development of vaccines using biotechnologies

Immunization can be one of the most effective means of preventing and hence managing animal diseases. In general, vaccines offer considerable benefits for comparative low cost, a primary consideration for developing countries. In addition, development of good vaccines for important infectious diseases can lead to reduced use of antibiotics, which is an important issue in developing countries (FAO, 2009d).

As described by Kurath (2008), biotechnology has been used extensively in the development of vaccines for aquaculture, and is applied at each of the three main stages of vaccine development i.e. a) identification of potential antigen candidates that might be effective in vaccines (where an antigen is a molecule, usually a protein foreign to the fish, which elicits an immune response on first exposure to the immune system by stimulating the production of antibodies specific to its various antigenic determinants. During subsequent exposures, the antigen is bound and inactivated by these antibodies) b) construction of a new candidate vaccine (where biotechnology tools can be used to produce different kinds of vaccines such as DNA vaccines, recombinant vaccines or modified live recombinant viruses. For example, a DNA vaccine is a circular DNA plasmid containing a gene for a protective antigenic protein from a pathogen of interest [see Kurath, 2008 for more details]), and c) assessment of candidate vaccine efficacy, its mode of action and the host response (where e.g. quantitative RT-PCR [see Section 2.4] can be used to examine the expression of fish genes related to immune responses).

Of the countries that responded to a recent OIE survey, 4 out of 23 and 7 out of 14 African and Asian countries respectively indicated that they produce or use animal vaccines derived from biotechnology, including experimental use as well as commercial release (MacKenzie, 2005).

## 2.6 Reproductive biotechnologies

A number of reproductive biotechnologies have been applied in developing countries to influence the number (and sex) of offspring from given individuals in fish and livestock populations.

### 2.6.1 Artificial insemination

In artificial insemination (AI), semen is collected from donor male animals, diluted in suitable diluents and manually inseminated into the female reproductive tract during oestrus (heat), to achieve

pregnancy. The semen can be fresh or preserved in liquid nitrogen and then thawed. Efficiency of AI can be increased by monitoring progesterone levels, e.g. using ELISA, to identify non-pregnant females, and/or by oestrus synchronization, where females are treated with hormones to bring them into oestrus at a desired time.

AI is widely used in developing countries (Chupin, 1992; FAO, 2007b). For example, in India 34 million inseminations were carried out in 2007 while about 8 million were carried out in Brazil (FAO, 2009c). For Africa, Asia and Latin America and the Caribbean regions, AI is mostly used for cattle production (dairy). Other species for which AI is used in all three continents are sheep, goats, horses and pigs. In addition, in Asia, AI is used for chickens, camels, buffaloes and ducks, and in Latin America and Caribbean regions for rabbits, buffaloes, donkeys, alpacas and turkeys. For the most part, semen from exotic breeds is used in local livestock populations. To a lesser extent, semen from local breeds is also used for this purpose. Most of the AI services are provided by the public sector but the contribution of the private sector, breeding organizations and NGOs is also substantial. In Africa and Asia, AI use is concentrated in peri-urban areas (FAO, 2007b; FAO, 2009c). Progesterone monitoring and oestrous synchronization have been applied in a number of developing countries. Applications of oestrous synchronization have been limited to some intensively managed farms where AI is routinely used (FAO, 2009c).

#### 2.6.2 Embryo transfer

Embryo transfer (ET) involves the transfer of an embryo from a superior donor female to a less valuable female animal. A donor is induced to superovulate (produce several ova) through hormonal treatment. The ova obtained are then fertilized within the donor, the embryos develop and are then removed and implanted in recipient females for the remainder of the gestation period. Alternatively, the embryos can be frozen for later use.

FAO (2007b) reports that 5, 8 and 12 countries use ET in Africa, Asia and the Latin America and the Caribbean region respectively. In the latter, ET is increasingly used by commercial livestock producers and the species involved are cattle (in all 12 countries) and alpacas, donkeys, goats, horses, llamas and sheep (in 1 to 3 of these 12 countries). In Brazil and Chile, private sector organizations are involved in providing the technology.

#### 2.6.3 Hormonal treatment in aquaculture

In the same way as female reproduction in livestock can be controlled by hormonal treatment, it is also an important tool in aquaculture where it is applied for 2 main purposes.

The first is to control reproduction of fish and shellfish, primarily to induce the final phase of ova production in order to synchronize ovulation and to enable broodstock to produce fish early in the season or when environmental conditions suppress the spawning timing of females. Implants or injection of the hormonal compound are used extensively in salmon farming (FAO, 2009d).

The second purpose is to develop monosex (single sex) populations, which can be desirable in many situations. This can be, *inter alia*, because one sex is superior in growth or has more desirable meat quality or to prevent sexual/territorial behaviour. For example, female sturgeon are more valuable than males because they produce caviar. Female salmon are the more valuable sex, because sexually precocious males die before they can be harvested and salmon roe has an economic value. Male tilapia are more desirable than females because they grow twice as fast. In many fish and shellfish species, sex is not permanently defined genetically and thus it can be altered in a number of ways, including treatment with sexual hormones such as testosterone or estrogen derivatives in early stages of development. To develop all-male tilapia populations, methyltestosterone can be used while monosex trout can be produced using androgens (FAO, 2009d).

#### 2.6.4 Sperm/embryo sexing



In livestock, to get offspring of a desired sex (e.g. females are preferred for dairy animals, males for beef animals), separation of X and Y sperm (e.g. based on staining DNA with a fluorescent dye) for AI and sexing of embryos (e.g. using specific DNA probes) can be used. Although these technologies are being developed and refined in a number of research institutions, they are not used at the field level in any of the developing countries, except China (FAO, 2009c).

## 2.7 Cryopreservation

Cryopreservation, referring to the preservation of germplasm in a dormant state by storage at ultra-low temperatures, usually in liquid nitrogen (-196 °C), can be used to preserve biological material (e.g. seeds, sperm, embryos) of crop, livestock, forest or fish populations for potential use in the future (Ruane and Sonnino, 2006a). The technology can be used for genetic improvement purposes and for management of genetic resources. In livestock, cryopreservation has been used in a number of developing countries for *ex situ* conservation of animal genetic resources, including Benin, Brazil, China, India and Kenya (FAO, 2009c). In fish, cryopreservation of embryos is not possible but sperm cryopreservation works for many species (Hiemstra et al, 2006) and has been used in carp, salmon and trout breeding, especially when the aim is to "refresh" populations that have gone through a bottleneck.

Considering crops and forest trees, about 90% of the 6 million plant accessions in genebanks, mainly crops, are stored in seed genebanks. However, storage of seeds is not an option for crops or trees that do not produce seed, such as banana, or that produce recalcitrant or non-orthodox seed (i.e. seed that does not survive under cold storage and/or the drying conditions used in conventional *ex situ* conservation), such as mango, coffee, oak and several tropical forest tree species. In these situations, as well as for long-term storage of seeds from orthodox species, cryopreservation offers an alternative strategy for *ex situ* conservation, although its routine use is still limited. Following plant cell, tissue or organ storage at low temperatures, plants can be regenerated. For various herbaceous (i.e. non-woody plants), hardwood (i.e. broadleaf, deciduous trees) and softwood species (i.e. coniferous trees), cryopreservation of a wide range of tissues and organs has been achieved. There is large scale application of shoot tip cryopreservation in fruit crop germplasm collections, such as in plum and apple. Seeds of most common agricultural and horticultural species can be cryopreserved (Panis and Lambardi, 2006; Ruane and Sonnino, 2006a).

## 2.8 Tissue culture-based techniques

Tissue culture refers to the *in vitro* culture of plant cells, tissues or organs in a nutrient medium under sterile conditions. It has been widely used for over 50 years and is now employed to improve many of the most important developing country crops (FAO, 2009a). There are a number of tissue culture-based technologies and they can be employed for a range of different purposes. Some of them, used with chromosome number manipulation, have already been described in Section 2.3. Others include:

### 2.8.1 Micropropagation

Micropropagation is the laboratory practice of rapidly multiplying stock plant material to produce a large number of progeny plants, using plant tissue culture methods. For instance, shoot tips of banana or potato are excised from healthy plants and cultivated on gelatinized nutrient media in sterile conditions (in test tubes, plastic flasks, or baby food jars), so that contamination with pests and pathogens is avoided. The obtained plantlets can be multiplied an unlimited number of times, by cutting them in single-node pieces and cultivating the cuttings in similar aseptic conditions. Millions of plantlets can be produced this way in a very short time. The plantlets are then transplanted in the field or nurseries, where they grow and yield low-cost, disease-free propagation materials, ready to be distributed to farmers (Sonnino et al, 2009). Even if healthy plants are not available initially, specific *in vitro* techniques can also be applied to produce disease-free propagation material.

Today, micropropagation is widely used in a range of developing country subsistence crops including banana, cassava, potato and sweetpotato; commercial plantation crops, such as oil palm, coffee, cocoa, sugarcane and tea; niche crops such as cardamom and vanilla; and fruit trees such as almond, citrus, coconut, mango and pineapple. Some of the many countries with significant crop micropropagation programs include Argentina, Cuba, Gabon, India, Indonesia, Kenya, Nigeria, Philippines, South Africa, Uganda and Vietnam (FAO, 2009a).

### 2.8.2 *In vitro* slow growth storage

Micropropagation procedures have been developed for over 1,000 plant species, many of which are today micropropagated commercially. The procedures include rapid multiplication, involving rapid growth and frequent subculture (regeneration) which is generally the objective of commercial micropropagation. Instead, the basis of successful *in vitro* storage of stock cultures is to increase the interval between subcultures by retarding the growth without any deleterious effects on the plants in culture. The strategy is used to conserve plant genetic resources and *in vitro* slow growth procedures can be used so that plant material can be held 1-15 years under tissue culture conditions with periodic subculturing, depending on the species. Normally, growth is limited using low temperatures often in combination with low light intensity or even darkness. Temperatures in the range of 0–5 °C are employed for cold-tolerant species and 15–20 °C for tropical species. Growth can also be limited by modifying the culture medium and reducing oxygen levels available to the cultures (Ruane and Sonnino, 2006a; Rao, 2004).

### 2.8.3 *In vitro* embryo rescue

Wide crossing (see Section 2.3) has only become possible by advances in plant tissue culture. A particular challenge was to overcome the biological mechanisms that normally prevent inter-specific and inter-genus crosses, as a high proportion of wide-hybrid seeds either do not develop to maturity or do not contain a viable embryo. To avoid spontaneous abortion, embryos are removed from the ovule at the earliest possible stage and placed into culture *in vitro*. Mortality rates can be high, but enough embryos normally survive the rigors of removal, transfer, tissue culture, and regeneration to produce adult hybrid plants for testing and further crossing (FAO, 2009a).

First-generation, wide-hybrid plants are rarely suitable for cultivation because they have only received half of their genes from the crop parent. From the other (non-crop) parent they have received, not only the small number of desirable genes, but also thousands of undesirable genes that must be removed by further manipulation. This is achieved by crossing the hybrid with the original crop plant, plus another round of embryo rescue, to grow up the new hybrids. This ‘backcrossing’ process is repeated for about six generations (sometimes more), until a plant is obtained that is almost identical to the original crop parent, except that it now contains a small number of desirable genes from the non-crop parent plant. Wide-crossing programs can take more than a decade to complete, although MAS and anther culture can be used to speed up the process (FAO, 2009a).

Embryo rescue has been used occasionally in forest tree species, but its application is likely to be limited to a small number of hybrids of interest, which are sufficiently close to produce a normal embryo but where embryo development *in vivo* is a limiting factor (FAO, 2009b).

## 2.9 Mutagenesis

This involves the use of mutagenic agents, such as chemicals or radiation, to modify DNA and hence create novel phenotypes. Induced mutagenesis has been used in crop breeding programs in developing countries since the 1930s. It also includes somaclonal mutagenesis, involving changes in DNA induced during *in vitro* culture. Somaclonal variation is normally regarded as an undesirable by-product of the stresses imposed on a plant by subjecting it to tissue culture. However, provided they are carefully controlled, somaclonal changes in cultured plant cells can generate variation useful to crop breeders (FAO, 2009a). In forestry, use of somaclonal variation has been a popular subject for

research, particularly during the 1980s, but the technology is generally seen to offer little for the genetic improvement of most major industrial forest tree species (FAO, 2009b).

Almost 3,000 new crop varieties have been developed and released by countries using mutation-assisted plant breeding strategies and an estimated 100 countries currently use induced mutation technology (FAO/IAEA, 2008; IAEA, 2008). Case studies from Kenya (wheat), Peru (barley), sub-Saharan Africa (cassava) and Vietnam (rice) are described in IAEA (2008).

In the livestock sector, mutagenesis has also been used in developing countries. The sterile insect technique (SIT) for control of insects (e.g. screwworm and tsetse flies) relies on the introduction of sterility in the females of the wild population. The sterility is produced following the mating of females with released males carrying, in their sperm, dominant lethal mutations that have been induced by ionizing radiation. This method is usually applied as part of an area-wide integrated pest management approach and has been applied in developing countries in the livestock sector as well as for the control of crop pests (FAO, 2009c). An estimated 30 countries use SIT against insect pests, including Chile and Peru (FAO/IAEA, 2008).

Mutagenesis is also extensively used to improve the quality of micro-organisms and their enzymes or metabolites used in food processing. The process involves the production of mutants through the exposure of microbial strains to mutagenic chemicals or ultraviolet rays. Improved strains thus produced are selected on the basis of specific properties such as improved flavour-producing ability or resistance to bacterial viruses (FAO, 2009e).

## 2.10 Fermentation

Fermentation is the process of bioconversion of organic substances by micro-organisms and/or enzymes of microbial, plant or animal origin. During fermentation, various biochemical activities take place leading to the break down of complex substances into simple substances and resulting in the production of a diversity of metabolites including simpler forms of proteins, carbohydrates, fats, such as sugars, amino acids, lipids, as well as new compounds such as antimicrobial compounds (e.g. lysozyme, bactericins); organic acids (e.g. lactic acid, acetic acid, citric acid); texture-forming agents (e.g. xanthan gum); and flavours (esters and aldehydes). Apart from the various new products that are yielded during fermentation, the process is widely known for its preservative benefits (Ruane and Sonnino, 2006b, chapter 6.1).

The new products that emerge following fermentation have been found to have potential for longer shelf lives, and they have characteristics quite different from the original substrates from which they are formed. Fermentation is globally applied to preserve a wide range of raw agricultural materials (cereals, roots, tubers, fruit and vegetables, milk, meat and fish, etc.). Commercially produced fermented foods which are marketed globally include dairy products (cheese, yogurt, fermented milks), sausages and soy sauce (Ruane and Sonnino, 2006b). Fermentation of sugars is also central to production of bioethanol from agricultural feedstocks (FAO, 2008b).

Certain micro-organisms associated with fermented foods, in particular strains of the *Lactobacillus* species, are probiotic i.e. used as live microbial dietary supplements or food ingredients that have a beneficial effect on the host by influencing the composition and/or metabolic activity of the flora of the gastrointestinal tract (Ruane and Sonnino, 2006b). They can also be used as feed additives for monogastric and ruminant animals, and have been applied for this purpose in China, India and Indonesia (FAO, 2009c).

In developing countries, fermented foods are produced generally at the household and village level, using traditional processes that are uncontrolled and dependent on spontaneous 'chance' micro-organisms from the environment. Modern fermentation processes employ the use of well constructed vessels (fermenters/bioreactors), with appropriate controlled mechanisms for temperatures, pH,

nutrients levels, oxygen tensions among others and also use selected micro-organisms and/or enzymes for their operations (FAO, 2009e; Ruane and Sonnino, 2006b).

### 2.11 Biofertilisers

Soils are dynamic living systems that contain a variety of micro-organisms such as bacteria, fungi and algae. Maintaining a favourable population of useful microflora is important from a fertility standpoint. The most commonly exploited micro-organisms are those that help in fixing atmospheric nitrogen for plant uptake or in solubilizing/mobilizing soil nutrients such as unavailable phosphorus into plant-available forms, in addition to secreting growth-promoting substances for enhancing crop yield. As a group, such microbes are called biofertilisers or microbial inoculants. They can be generally defined as preparations containing live or latent cells of efficient strains of nitrogen-fixing, phosphate-solubilizing or cellulolytic micro-organisms used for application to seed or soil with the objective of increasing the numbers of such micro-organisms and accelerating certain microbial processes to augment the availability of nutrients in a form that plants can assimilate readily (Motsara and Roy, 2008). Biofertilisers have been used in a number of developing countries, such as Kenya and Thailand, often involving nitrogen-fixing *Rhizobia* bacteria (Sonnino et al, 2009).

### 2.12 Biopesticides

Living organisms that are harmful to plants and cause biotic stresses are collectively called pests, and they cause tremendous economic damage to plant production worldwide. Biopesticides are mass-produced, biologically based agents used for the control of plant pests. They can be living organisms, such as micro-organisms, or naturally occurring substances, such as plant extracts or insect pheromones. Micro-organisms used as biopesticides include bacteria, protozoa, fungi and viruses and they are used in a range of different crops (Chandler et al, 2008).

For example, different biopesticides are available for controlling locusts. As an illustration, a biopesticide containing spores of the fungus *Metarhizium anisopliae*, was used to control a migratory locust infestation in an FAO project in 2007 in Timor-Leste. Surveys revealed that an area of about 20,000 hectares was infested with gregarious nymphs and that there was a serious threat to the rice crop. The target area was considered unsuitable for chemical spraying because of high density human settlement and many water courses, so the infestation was treated with the biopesticide, targeting flying swarms using a helicopter, spraying in a time period of over one month (FAO, 2009f). Note, biopesticides generally have a slow action compared to conventional chemicals and, for that reason, the latter are preferred if crops are under immediate threat.

## 3. Specific Points About This E-mail Conference

The general aim of the e-mail conference is to bring together and discuss relevant, often previously un-documented, past experiences of applying biotechnologies at the field level (i.e. used by farmers for commercial production) in developing countries, ascertain the success or failure (be it partial or total) of their application, and determine and evaluate the key factors that were responsible for their success or failure. The conference does not cover experiences in developed countries.

### 3.1 Issues to be addressed in the e-mail conference

For any one (or combination) of the biotechnologies described in Section 2, considering its application at the field level in one of the different food and agricultural sectors (crops, livestock, forestry, fishery or agro-industry), in any particular developing country or region, and in any specific time period over the past 20 years:

- provide an overall assessment of the experience of applying the biotechnology i.e. was it a success or failure, partial or full (and provide a justification for this assessment)
- based on this, describe some of the key features that determined its partial or complete success (or failure)

- if possible, indicate how transferable these results might be to other a) developing countries/regions b) biotechnologies and c) food and agricultural sectors
- indicate any lessons that can be drawn from this experience that may be important for applications of agricultural biotechnology in developing countries in the future

### 3.2 Defining success and failure

When considering a certain situation where a biotechnology was implemented in a specific developing country, sector and time period, and attempting to assess it as a full or partial success (or failure), a number of different aspects can be taken into consideration, such as any potential impacts its application had of a socio-economic, cultural, regulatory, environmental, agro-ecological, nutritional, health and hygiene, consumer interest and perceptions, sustainable livelihoods, equity, technology transfer or food security nature. For example, if we consider the use of a reproductive technology such as artificial insemination in a certain livestock species (e.g. dairy cattle) in a given developing country, some of the factors which might influence whether we would consider it to be a success or failure could include the impact that applying the biotechnology had on:

- milk production (the trait of main interest)
- other traits, such as cow fertility and health, that can be indirectly affected (often negatively) by improvements in milk production
- trade (e.g. did use of the biotechnology result in surpluses that led to creation of new trade opportunities? Alternatively, did its use result in closure of some existing markets, e.g. due to regulatory issues?).
- economic returns to the farmer, considering the increased financial returns from increased milk yields as well as any additional costs from using the biotechnology, such as the cost of inseminating the cow, any additional feed or veterinary bills, etc.
- food security (e.g. was more milk produced, leading to greater food security?)
- equity (e.g. was use of the biotechnology restricted to already-rich farmers or did its use also extend to the more food-insecure smallholders; also who gained from sale of the biotechnology itself ? [e.g. were the AI services provided by a foreign multinational company or by a local farmers co-operative])
- consumer interests (did use of the biotechnology produce a negative consumer reaction, resulting in reduced milk consumption?)
- genetic resources (e.g. if AI was used to cross local females with semen from bulls of developed countries, did it result in erosion of valuable genetic resources in developing countries)
- technical aspects related to applying the biotechnology (e.g. did it work properly, was much training/equipment needed for people to use it?)
- any unexpected impacts of using the biotechnology.

The number of potential factors that could influence the overall assessment of the biotechnology as a success or failure (partial or complete) is therefore quite large and, for a given case, some of the factors might be negative and others positive. Thus, the fact that a certain biotechnology has been used (and maybe continues to be used) does not mean *per se* that it has been a success, although in certain cases, it may be considered as an indicator of success.

A major hurdle to determining fully whether specific applications of biotechnology have been a success or failure is that there is normally a lack of solid, scientifically sound data and documentation about the impacts of their application on people's livelihoods and their socio-economic conditions etc. (Sonnino et al, 2009). Indeed, one of the aims of this e-mail conference is to try and get a better insight and more information on such areas.

### 3.3 Covering GM versus non-GM biotechnologies

The conference will be moderated and one of the Moderator's main tasks is to ensure that all of the biotechnologies as well as all of the food and agricultural sectors are adequately covered in the conference. As anyone following this area knows, the topic of genetic modification, and GMOs, is one of major interest and has been the object of a highly polarized debate, particularly concerning GM

crops. One of the consequences of this is that the actual impacts and the potential benefits of the many non-GM biotechnologies have tended to be neglected. However, to learn from the past regarding applications of agricultural biotechnologies in developing countries, the entire range of biotechnologies should be considered as there may be many specificities related to any particular biotechnology tool, regarding aspects such as its financial, technical and human capacity requirements, its purpose (e.g. genetic improvement, genetic resources management or disease diagnosis), its potential impacts etc. For this reason, we ask participants to ensure that all the biotechnologies and all the food and agricultural sectors are covered adequately. In addition, regarding GMOs, discussion in the conference should not consider the issues of whether GMOs should or should not be used *per se* or the attributes, positive or negative, of GMOs themselves. Instead, the goal is to bring together and discuss specific experiences of applying biotechnologies (including genetic modification) in the past in developing countries.

### 3.4 Submitting a message

Before submitting a message, participants are requested to:

- a) ensure that it considers the issues mentioned above in Section 3 and the biotechnologies mentioned in Section 2
- b) limit its length to 600 words
- c) read the Rules of the Forum and the Guidelines for Participation in the E-mail Conferences. These were provided by e-mail when joining the Forum, and they can also be found at <http://www.fao.org/biotech/forum.asp>. One important rule is that participants are assumed to be speaking in their personal capacity, unless they explicitly state that their contribution represents the views of their organization.

When submitting their first message, participants should introduce themselves briefly, providing also their full address at the end of the message.

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ABBREVIATIONS: AFLP = Amplified fragment length polymorphism; AI = Artificial insemination; Bt = *Bacillus thuringiensis*; ELISA = Enzyme-linked immunosorbent assay; ET = Embryo transfer; FAO = Food and Agriculture Organization of the United Nations; GMM = Genetically modified micro-organism; GMO = Genetically modified organism; IAEA = International Atomic Energy Agency; MAS = Marker-assisted selection; OIE = World Organisation for Animal Health; PCR = Polymerase chain reaction; RAPD = Random amplified polymorphic DNA; rBST = recombinant bovine somatotropin; RFLP = Restriction fragment length polymorphism; RT-PCR = reverse transcriptase PCR; SIT = Sterile insect technique.

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