BIOTECHNOLOGY AND AGROBIODIVERSITY

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Summary

Biotechnology and agrobiodiversity strongly interrelate. Mutual influences traditionally occurred and shaped our current agriculture. However, the influence of biotechnology on agrobiodiversity has recently strongly increased as a result of the enormous developments in modern biotechnology, whether in *in vitro* technology, molecular markers or genetic modification.

In a sense, these effects are not really novel but fit into the longer-term industrialization of agriculture which started in the developed countries a century ago. However, biotechnology has accelerated and strengthened such effects.

These effects have been both positive and negative and will probably continue to be so.

The balance between the positive and negative effects to a large extent depends on strategic choices and decision making by private companies, governments and their research institutions, and community organisations.

Among the outstanding positive effects of biotechnology on agrobiodiversity may be regarded as,

- the contribution to more efficient *ex situ* management of genetic resources, through the use of *in vitro* tissue culture and cryopreservation techniques
- the use of molecular markers to analyse the genetic diversity present in *ex situ* collections and in on-farm and *in situ* populations of crops and domestic animals and their wild relatives
- the potential to protect the markets of traditional and regional genetic varieties by monitoring the integrity of market produce offered as such
- the use of molecular markers to improve the breeding of new varieties with the help of genetic resources, increasingly of markers which directly relate to specific traits
- the potential to improve the nutritional quality of our food and the sustainability of our agricultural production by exploiting genetic resources not available to traditional breeding because of sexual barriers.

Major negative effects include:

- the contribution of biotechnology to a further narrowing of global crop and animal improvement efforts on a small number of species and a limited intraspecific genetic diversity, due to the high investments needed for biotechnological applications and the consequent access of biotechnology to a small number of players
- the increasing reliance on *ex situ* genetic resources as opposed to the maintenance of agrobiodiversity *in situ*
- the growing privatisation of genetic resources through intellectual property rights and the exclusion of these genetic resources from the genetic pools available for further breeding

Agrobiodiversity has become an increasingly useful source for crop and animal improvement since biotechnology has widened and facilitated the potential use of genetic resources. Useful traits can be much more easily linked to specific genes and gene complexes, and such genes can be transferred across traditional genetic barriers. These options have raised awareness about the value of genetic resources, but not on the need to maintain our genetic diversity *in situ* as part of wider agrobiodiversity.

Awareness raising and public discussions will continue to be needed to correct for the current imbalance between biotechnology and agrobiodiversity.

1. Introduction

For centuries traditional biotechnological processes have been used to contribute to agricultural production (see also - *Traditional Plant Breeding for Yield Improvement*

and Pest Resistance) and food processing (see also - Food Fermentation and Processing). However, as a result of the recent advances in molecular biology and genetics, existing biotechnological applications have been refined and many new biotechnological tools have been developed. Modern biotechnology has left the laboratory and reached the market place. The impact of biotechnology on agricultural production and food processing has accordingly increased. Traditional and modern biotechnology can be regarded as a continuum, with modern biotechnology characterized as laboratory-based and resource-intensive. As a consequence of its biological basis, the use of traditional and modern biotechnology shows strong interactions with agricultural biodiversity: biotechnological applications are based on biodiversity, and in turn influence biodiversity (see also– *Biotechnology in the Environment: Potential Effects on Biodiversity*).

Various definitions of biological diversity or biodiversity (see also- Biodiversity: The Impact of Biotechnology), agricultural biodiversity or agrobiodiversity, and biotechnology have been given. Now widely accepted are the definitions in the Convention on Biological Diversity, agreed in 1992, for biological diversity and biotechnology. Biotechnology is defined as 'any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use.' It is clear that this definition encompasses both traditional and modern biotechnological applications. Biological diversity is defined as 'the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems.' Questions of diversity within species, i.e. genetic diversity, can be addressed at the species, population, and within-population levels. Thus, this definition distinguishes three levels of integration with increasing complexity. Agricultural biodiversity, also known as agrobiodiversity, forms a subset of total biodiversity. It refers to biodiversity related to agriculture and can be described as 'the variety and variability amongst living organisms (of animals, plants, and microorganisms) that are important to food and agriculture in the broad sense and associated with cultivating crops and rearing animals and the ecological complexes of which they form a part.' It includes the diversity found in farming systems as well as their surroundings to the extent that the latter influences agriculture. Food processing technologies – strictly speaking – are not covered by this definition, and will not be discussed, although the relationship between biotechnology and agrobiodiversity also largely holds for these technologies (see also-Biotechnological Applications for Food Production).

The interactions between biotechnology and agrobiodiversity are manifold. Traditional biotechnological applications in particular make use of microbial organisms. They include:

- composting, the accelerated microbial degradation of organic matter (see also -Composting agricultural and industrial wastes);
- nitrogen fixation, based on the ability of bacterial symbionts of leguminous plants to fix atmospheric nitrogen (see also - *Nitrogen fixation biotechnology*);
- fermentation, generating alcoholic beverages and dairy products and preserving a large variety of meat and plant products (see also - *Industrial Biotechnology*);

• ethnoveterinary practices aimed at protection of domestic animals against infectious diseases (vaccine production).

For these applications, specific microbial strains have co-evolved and often been selected. In other words, biodiversity has been exploited to allow for these traditional biotechnological applications. Modern biotechnology has developed much more refined tools to select and generate optimal microbial organisms for a larger set of applications, and each of these involves the use of specific traits generated and expressed in the microbial domain.

These applications, as well as applications making use of plant and animal biodiversity, have contributed to the shaping of agricultural production. Cereals and pulses have been intercropped in all Centers of Origin of agricultural crops, an essential combination to guarantee high yields. Other crops, such as soy bean and enset (Ethiopia), have gained importance because protocols were developed to ferment the harvested products and allow for prolonged food storage. Ruminants (cattle as well as sheep and goats) have been selected for milk production concomitantly with the development of milk fermentation technologies.

Modern biotechnology has already influenced the genetic diversity of crops (see also -Transgenic Plants and Crop Protection through Pest-resistant Genes) and animals cultivated and raised in the fields by allowing for the rapid and wide-spread introduction of desired starting material through in vitro technology. It is now on the brink of more profoundly changing our agriculture, in particular by the generation of novel crop varieties containing traits which could not be incorporated before and altering our farming practices. In this way, biotechnology has also changed and will further change our agricultural biodiversity. It can improve our production systems and the diversity of products we desire. The question to answer is whether agricultural biotechnology will be able to change the current, often negative image of modern agriculture into a new image which stands for a more sustainable way of dealing with resources, people and problems, or is going to intensify current problems in agriculture concerning sustainability and biodiversity. Modern biotechnology also has a potential or actual influence on natural ecosystems, either by allowing changes in agriculture which affect natural biodiversity or by directly influencing natural biodiversity itself. These developments are now often discussed in the framework of biosafety policies (see also Biosafety in biotechnology; Biotechnology in the environment: Potential effects on biodiversity).

This introduction so far attempts to stress the continuum in the interactions between traditional and modern biotechnology and biodiversity. Also from another perspective, a continuum characterizes the influence of biotechnology on (agro) biodiversity in particular and agricultural practices in general. From ancient times onwards, farming practices, including animal and plant breeding, have profoundly influenced our agro-ecosystems and the biodiversity these contain, and thus this influence is not the prerogative of biotechnology but of technologies applied in agriculture in general. Impacts on biodiversity may not be different in nature, only in degree, from those of our traditional or conventional practices.

The term genetic resources refer to a resource-centered view on biodiversity.

Accordingly, it is often stated that loss of biodiversity means loss of capital and potentially useful resources. The immediate relevance of agrobiodiversity concerns food production and the prime incentive to preserve agrobiodiversity is based on economics (see also *The Economics of Agrobiotechnology*). Although agriculture is both the predominant land-use system on earth and the largest global user of biodiversity, agrobiodiversity is a surprisingly minor topic in the global biodiversity debate.

The following text further elaborates the interactions between biodiversity and the applications of biotechnology. In particular, it focuses on the effects of the rapid developments in modern biotechnology on these interactions, both beneficial and detrimental. It deals with the use of biodiversity in biotechnological applications, and with the subsequent effects of these applications on the biodiversity in farming systems and natural environments. Although a substantial part of research is currently devoted to the diversity of beneficial and noxious insects, microorganisms and other soil organisms, we have limited ourselves to the interface between agrobiotechnology and plant and animal biodiversity relevant to agro-ecosystems.

2. Technical aspects of agricultural biotechnology at the interface with agrobiodiversity

Modern biotechnology can be defined as all biotechnology that involves a laboratory phase in its development or application. The term stands for several different laboratory-based technologies that are used either independently or in combination with each other. These technologies are *in vitro* technology, marker technology and gene technology. More recent technological advances, commonly referred to as 'genomics', allow the application of former technologies in an (ultra)high-throughput context. As a consequence, genomics will further speed up the pace of (agro)biotechnological innovations. We will give a brief overview of the tools of modern agricultural biotechnology as applied to plants and animals. Although microbes are an integral part of agricultural systems, and microbial biotechnology is well developed, its application is predominantly in processing and food production. For an in-depth discussion of the tools briefly explained below, the reader is referred to related chapters in this theme (see *- Industrial Biotechnology*).

2.1 In vitro technologies

Modern plant biotechnology is based on hydroponics, the capacity to grow plants without a soil substrate in an aqueous solution of macro and micronutrients and light. Hydroponics has developed into a major technology for the mass propagation of high-value vegetable crops. It also formed the basis for the development of plant cell and tissue culture techniques. Micropropagation allows the regeneration of complete new plants from leaves or shoot apexes; free from diseases, notably viruses, and thus a rapid clonal propagation of elite plant material. This is usually but not necessarily carried out in specialized laboratories. In particular the micropropagation of ornamentals and trees has become an important economic enterprise. The capacity of a single cell to regenerate into whole plants, is known as cell totipotency. It signifies the ultimate form of micropropagation. As an alternative application, *in vitro* somatic embryogenesis can be used for rapid propagation of plant material, and in various cases encapsulated

embryos are replacing seeds. Also, the culturing of specialised plant cells in the form of mass cell suspensions has developed into a technology for the production of specialty chemicals. Finally, *in vitro* technology has been instrumental in the development of technologies as protoplast fusion, embryo rescue, mutagenesis and others that are part of modern plant breeding, as well as in the development of cryopreservation techniques, the storage of living cells at ultra-low temperatures (see also - *Plant Cell Culture*).

Storage of cells, tissues and organs of animal origin has also been achieved by cryopreservation (see also - *Mammalian Cell Culture*).

Micropropagation of plant material allows the production of millions of clonal progeny that are essential identical. Nowadays, more than a thousand plant species are being propagated in tissue culture. The obvious advantage is that elite material can be quickly multiplied without changing the desirable genetic constitution of the material. Obtaining disease-free plants is another major advantage of the tissue culture approach. However, micropropagation in tissue culture also suffers from somaclonal variation, due to rearrangements and mutation of the DNA during the maintenance of cells in tissue culture. Tissue culture-induced somaclonal variation can also be exploited as a new source of genetic variation for crop improvement. This way, tissue culture, as well as mutagenic treatments, can contribute to a limited extent to an increase of diversity (see also - *Traditional Plant Breeding for Yield Improvement and Pest Resistance*).

In contrast to plant cells, animal cells are generally not totipotent. Although individual cells can be propagated in culture, regeneration of such cells into a new, fully developed animal is not known. What has been realized is the induction of directed differentiation (based on multipotentiality) of somatic mammalian and other animal cells (see also–*Transgenic Technologies for Animals as Bioreactors*).

In its effects, artificial insemination can be regarded as the animal equivalent of plant micropropagation. More recent and advanced developments have resulted *in vi*tro fertilisation combined with embryo transfer, widening the options for rapid propagation of desired genotypes.

2.2 Genetic modification and the sourcing of genes

In vitro technology formed a prerequisite for the development of genetic modification of plants. Genetic modification of plants combines techniques for plant tissue culture, techniques for cloning, *in vitro* amplification (PCR), and transfer of DNA, either by the use of the soil bacterium *Agrobacterium tumefaciens* as a vector, through electroporation or by the use of a particle gun. It is based on the ability to change the genetic constitution of a single cell and regenerate a new plant from that single cell (see also - *Genetic engineering of plant cells*) The aims of plant genetic modification are manifold. The technology can be used to transfer a gene from a wild relative simply to speed up breeding, but it can also be used for the transfer of genes into the plant gene pool that could not be introduced into plant genomes by other means.

In 1999 more than 70 transgenic crop varieties (see also - *Transgenic plants*) were registered for commercial cultivation. The crops involved include corn, soybean, rapeseed, tomato, tobacco, potato, chicory, papaya, pumpkin and clover. In 2005,

biotech soybean continued to be the principal biotech crop, occupying 54.4 million hectares (60% of global biotech area), followed by maize (21.2 million hectares at 24%), cotton (9.8 million hectares at 11%) and canola (4.6 million hectares at 5% of global biotech crop area). During the first decade, 1996 to 2005, herbicide tolerance has consistently been the dominant trait followed by insect resistance and stacked genes for the two traits (James, 2005). In the future diversification may be expected not only from the private sector, but also from the (international) public sector. The CGIAR Challenge programme Generation focuses on drought tolerance in cereals, legumes and clonal crops. Genetic modification efforts of the M.S. Swaminathan Foundation regard combating drought and salinity in locally adapted rice and wheat varieties, whereas the Indian Initiative on Crop Biofortification focuses on bio-availability and bio-efficacy with respect to iron, zinc and phosphor in rice, wheat and maize.

Beneficial applications of genetic modification in crop breeding and food production may include the following short-term options:

- Harnessing natural fungal resistance expressed in the seed phase for resistance in other phases of the plant growth cycle
- Developing non-toxin based insect resistance traits (e.g. hairiness)
- Changing seasonal conditions for plant growth, allowing the shift from winter crops to spring crops in moderate climates and allowing fallow land periods
- Developing crops with higher levels of insect tolerance

In order to improve or change a plant variety by genetic modification, a biotechnologist requires genes to code for the new character. As a consequence, biotechnology has a great need for well-characterized genes, preferably available as pieces of DNA or cDNA (= cloned RNA). Characteristic for genetic modification is that the origin of the gene of interest is not important. DNA is virtually independent of the organism from which it originates. The attitude of plant biotechnologists to plant improvement is often as straightforward as ambitious: for any trait desired, somewhere there will be an organism providing the genes that are able to do the job. The challenge is to find those organisms, to identify the gene or genes involved, and to transfer those genes into a suitable plant. Thus bioprospecting, the identification and evaluation of properties in organisms for potential exploitation, is currently becoming a major input in (plant) biotechnology. The suitable organism may be a plant, but may also be a bacterium or a whale: the concept of 'wild relatives' is of no relevance to biotechnology. The interest of biotechnology in genetic diversity is therefore unlimited and transcends the scope of gene bank collections or populations being conserved on-farm. Botanical gardens, national parks or pristine nature may be as useful, hence valuable, as the diversity in local farming communities. Any organism in any ecosystem may contain genes that are or may become of use for any application. All organisms should therefore be conserved. Biotechnologists are in a prime position to recognize the importance of genetic resources. Here the key issue is documentation. Which genes or properties or compounds are present in which organism; what is the function of these genes and compounds? And where is the organism and the relevant DNA or cDNA likely to be found?

Breeders and curators of plant genetic resources collections traditionally distinguish the

primary, secondary and tertiary gene pools, based on the ease by which germplasm of different plants can be recombined. In line with this terminology, biotechnology relies on what could be considered the 'quaternary gene pool', encompassing all genetic material that occurs in nature but cannot be introduced into a plant by any sexual means. To fulfil a role in current plant biotechnology and plant genetic modification, genebanks might consider storing DNAs and cDNAs from a much wider range of species, forming part of this quaternary genepool. Furthermore, biotechnological tools might enable the much wider exploitation of indigenous knowledge on the properties of plants and microbial organisms which form part of the quaternary genepool, in food production and beyond. In the near future, biotechnology may also be able to create new diversity that could be considered a 'fifth-level gene pool'. Examples of novel diversity created in the laboratory include the generation of new genes that encode proteins with improved functions by DNA shuffling, the random recombination of existing genes. Random shuffling of genes can be considered as the molecular equivalent of processes resulting in heterosis. However, this technique creates genetic diversity that does not necessarily have a counterpart in nature.

Genetic modification of animals is based on a combination of technologies (see also -*Genetic engineering of animal or mammalian cells*), as in the case of plants. It involves the genetic information and reproductive tissues that generate new organisms: nuclei, fertilized eggs, embryos, gonads or embryogenic stem cells, as well as the totipotent cells originating from the inner cell mass of the early blastula stage. Embryonic stem cells can be cultured *in vitro*, engineered and introduced in the inner cell mass of a suitable mammalian embryo. Gene transfer in animal genetic engineering is achieved either by microinjection, lipofection or electroporation.

In principle, new techniques involving inter-species somatic cell cloning and interspecies nuclear transfer technology allow for more efficient rescue of endangered animal species.

Discussions about the application of animal biotechnology run parallel to those on the application of plant biotechnology, but bio-ethical considerations are much more prominent in applications involving animals.



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Biographical Sketches

Bert Visser is a molecular biologist by training. He is the director of the Centre for Genetic Resources, The Netherlands, which holds the mandate for the implementation of the Dutch plant genetic resources programme, and maintains a gene bank of 20 different crops. He is involved in policy development of PGR and in genetic resources management aspects, and participates in various international programs on this issue. He is particularly interested in complementarities between ex situ and in situ conservation approaches.

Jan-Peter Nap is a senior molecular biologist working on the predictability of transgene expression in genetically modified plants and bio safety aspects of transgenic plants.