1. Biotechnology

Biotechnology is a relatively recent term that appeared for the first time around 1960. Its origin is the Greek word *Bio* meaning life, and *Technology*, which appeared in the French language in 1656 meaning the "study of tools, machines and raw materials". Although the etymology is fairly precise, its definition is a little more wide-ranging, and even subjective at times (Bhojwani, 1990).

The use of living organisms and their products for commercial purposes is a broad definition. The first wine and bread makers could have been described as biotechnologists before the term was coined. A more restricted understanding of the term biotechnology would link it to the achievements of the last sixty years, including all *in vitro* culture techniques and the many facets of molecular genetics, such as gene cloning, sequencing and genetic engineering. Similarly, there are two possible definitions of the term plant biotechnology: one, *in sensu lato* (or traditional), defines plant biotechnology as human intervention on plant material by means of technological instruments in order to produce temporary effects (Fig. 1).
Figure 1: (Traditional definition) Plant biotechnology as human intervention on plants by means of technological instruments in order to produce temporary reactions (from the Authors).
The other definition, *in sensu stricto* (or modern), defines plant biotechnology as human intervention on plant material by means of technological instruments in order to produce permanent effects (which are transferable to the progeny), and includes genetic engineering and gene manipulation to obtain transgenic plants (Fig. 2).

**Figure 2:** (Innovative or advanced definition) Plant biotechnology as human intervention on plants by means of technological instruments in order to produce permanent reactions and then transferable to the progeny (from the Authors).

Plant genetic engineering is used to produce new inheritable combinations by introducing external DNA to plant material in an unnatural way. The results are Genetically Modified Plants (GMPs) or transgenic plants.
The key instrument used in plant biotechnology is the Plant Tissue Culture (PTC) technique which refers to the *in vitro* culture of protoplasts, cells, tissues and organs. It consists of culturing tissues
or cells in totally artificial conditions; unfortunately, not all factors can always be fully controlled and it is not always possible to reproduce every *in vitro* procedure. Nevertheless, the PTC technique involves the following steps:

- the growth of microbe-free plant material in aseptic (sterile) conditions, such as a closed test tube or container. Great care is taken to maintain strict aseptic conditions during the manipulation and culture of plant material;
- the use of sterile and well-known media because many inorganic and organic components are involved in the *in vitro* culture of plant material;
- the use of a growth room in which the environment (light and temperature) is controlled (Fig. 3).
There has been incredible progress in the development of PTC technology over the past four decades. In fact, although at the beginning of the 20th century Haberlandt introduced the culture of single plant cells, his experimentation never proved successful. After attempts by White and Gautheret, the person who initiated meristem culture technology was Morel in 1960. Steck and his colleagues were the pioneers of the *in vitro* PTC technique for production of phytochemicals in 1970, and in recent years the cell culture system has been used to produce compounds of medicinal importance. Studies related to plant regeneration from cell cultures were started by Reinert in 1959 and somatic embryos were obtained in 1993. In 1970, *in vitro* protoplast technology, an instrument for plant breeding, had resulted in the cell division stage. The technology became innovative when gene transfers between *in vitro*-cultured plant material were carried out (Augée *et al.*, 1995).
To carry out the *in vitro* PTC technique properly, a laboratory and specific equipment are required. Nowadays PTC is the main instrument for plant biotechnology and is used in:

- plant propagation or vegetative multiplication or cloning (Micropropagation), in addition to traditional methods used in nursery activity (grafting, cutting, layering, division) (Fig. 4),

**Figure 4**: *In vitro* culture as a plant biotechnology instrument for vegetative propagation (from the Authors).
- plant material and genotype conservation or plant material storage (Fig. 5),

**Figure 5:** In vitro culture as a plant biotechnology instrument for plant material conservation (from the Authors).
- improvement in plant health quality (plant recovery from virus) (Fig. 6),

**Figure 6:** *In vitro* culture as a plant biotechnology instrument for plant health recovery (from the Authors).
- production of useful substances in pharmacology, agriculture and industry (Fig. 7),

**Figure 7:** *In vitro* culture as a plant biotechnology instrument for useful substance production (from the Authors).
- plant breeding by somaclonal variation, selective pressure, the haploidy method, protoplast fusion and transfer of foreign DNA (Fig. 8),

**Figure 8:** *In vitro* culture as a plant biotechnology instrument for plant breeding (from the Authors).

- research in plant biochemistry, plant physiology and plant morphology.

 Crossing and selection are the methods traditionally used in plant breeding programmes. For about half a century, the *in vitro* culture technique has been widely used in plant breeding, and also in genetic engineering.

Initially the *in vitro* PTC technique was used to support the traditional or conventional breeding procedure in order to save time and/or to accelerate the evaluation of new genotypes. For some decades now, the use of this technique in breeding programmes has been increasing and it is actually considered more as a follow-on method than to back up traditional ones. As a breeding technique, *in vitro* culture can be divided into two groups (Taji *et al.*, 2002):

1. the first group is defined as traditional or conventional because it induces variation or mutation in plant tissue by chemical and/or physical processes, by choosing the initial explant and the
culture conditions (nutritive and environmental). In these procedures there is no addition of external or new DNA to plant tissue and, consequently, variation is due to the new combination of DNA contained within the tissue. At present, three principal techniques are available to induce variation or mutation in plants:
- protoplast fusion,
- haploidy methods,
- somaclonal variation.
Nevertheless, two types of somaclonal variation are possible: heritable and epigenetic. The first is stable through the sexual cycle or repeated asexual (vegetative) propagation and may be defined as a mutation because it results in heritable alteration in the genotype due to a change in the structure of the genetic material of the involved plant tissue (i.e., DNA base sequence). Epigenetic variation is unstable, even when asexually propagated, and disappears in the progeny or when the cause of variation stops.

2. the second group of in vitro breeding techniques includes those in which the new genetic combination is the consequence of deliberate and unnatural insertion of external DNA into the original plant genome by technological instruments. In other words, foreign DNA is inserted into plant material, which becomes transgenic. To transport the DNA from donor biological material to host plant tissue, different biological vectors can be used (i.e., Agrobacterium, plasmids, virus) as well as chemical and physical methods (biolistic system, protoplast fusion, somatic hybridisation, electroporation, electrofusion, microinjection). Regenerated plants from the new genome combination obtained by these innovative in vitro culture techniques, also called genetic engineering, are known as GMPs, which are included in the larger category of GMOs (Genetically Modified Organisms).

Without considering the vector used to transfer DNA between biological (plant) material, the general procedure for obtaining and using transgenic plants involves the following steps (Rosu, 1999):
- gene location and isolation from donor,
- gene cloning,
- gene insertion into the host plant tissue,
- transgenic plant regeneration,
- genetic characterisation and marker-assisted selection,
- placing on the market procedure (including effects on the environment and on animal and human health),
- commercial development.
Therefore it is first necessary to have an effective procedure for plant regeneration from the modified callus of the species involved in the breeding programme. In some cases, plant regeneration from tissue with recombinant DNA is very difficult.

In 2004, approximately 8.2 million farmers from all over the world grew genetically modified crops on more than 81 million hectares. The United States alone cultivated 47.6 million hectares. Genetically modified crops cover about 85-90% of soybeans, and 80% of cotton. Argentina ranks second, with a surface of 16.2 million hectares. In 2004, Canada cultivated 5.4 million hectares with GMOs, Brazil 5 million hectares, and China 3.7 million hectares. The rest of the 'transgenic surface' was cultivated by 12 other countries, — South Africa, Australia, India, Romania, Spain, Uruguay, Mexico, Bulgaria, Indonesia, Columbia, Honduras and Germany. In European countries, very little land is used to grow genetically modified crops (0.2%). This is largely the result of consumer rejection of GMOs (James, 2005).

*In vitro* PTC is an extraordinary tool for physiology and biology studies. Organs and/or tissues can be separated from the influence of other parts of a plant and also from environmental constraints. Furthermore, *in vitro* studies are more responsive to chemical stimuli. Bio-tests and bioassays carried out using the PTC technique could be considered an efficient method for evaluating:

- agrochemical disturbance to crops (i.e., herbicides, growth regulators, pesticides),
- effects of the environment on plant growth and metabolism,
- response of plants to various stimuli.

2. **Agricultural biodiversity and transgenic plants**

Agricultural management has a considerable impact on biodiversity. Many components of biodiversity directly depend on, or co-exist with, agricultural systems. Agricultural production should be intertwined with biodiversity conservation.

Agriculture is an important source of biological diversity through the preservation of various genes, biotypes, populations and species adapted to different habitats. Agricultural biodiversity or "agrobiodiversity" includes all the components of biological diversity — plants, microorganisms, animals — that are important for food and agriculture. Over time, the preservation of the genetic diversity of the grown species has led to a permanent increase in agricultural production. Genetic diversity is the basis for the adaptability of species to the environment. Rich genetic diversity results in the creation of new plant varieties that use the diversity of the environment and thus meet human demands for food, fibres, medicine, fodder and energy, etc. (IPGRI, 1993; Isik *et al.*, 1997).
The abandonment of agricultural land, a phenomenon occurring in several European countries, may become a threat to regional biodiversity, as various species, particularly birds, live on cultivated land. Biodiversity in general is threatened by such factors as:

- global climate change,
- habitat destruction or fragmentation,
- agriculture and forestry exploitation,
- excessive exploitation of natural resources,
- pollution of environmental components — soil, water, air,
- introduction of new species — the lack of co-evolution of species may endanger indigenous species.

Preservation of agrobiodiversity is an important part of EU agricultural policy, and is included in several Community programmes stipulating immediate measures to end biodiversity losses by 2010. An action plan in favour of agricultural biodiversity was initiated in 2001. As part of the EU’s Common Agricultural Policy and Agenda 2000 (adopted during the Berlin EU Council in spring 1999 and providing the framework for the Common Agricultural Policy until 2006), the plan includes the following: promotion of agricultural practices and systems that value the environment and favour biodiversity both directly and indirectly; support of sustainable agriculture in biodiverse rich areas; preservation and ecological reclamation of affected areas, and promotion of initiatives for the conservation of animal species/varieties and local or endangered plants. The action plan stipulates that measures for biodiversity conservation should be backed by scientific research and education.

The development of agricultural biotechnologies towards the end of the 20th century, and the introduction of genetically modified organisms (GMOs) on the market, are an important source of agricultural biodiversity. By means of biotechnology, organisms may become better adapted to the environment or may comply with certain consumer demands (e.g., nutritive value or chemical composition).

Genetically modified (transgenic) plants are plants into which certain genes (with the wanted traits) have been inserted using modern genetic engineering techniques which are more targeted than traditional breeding methods.

Thus, new plant varieties or hybrids can be obtained, with, for example, the following characteristics: resistance to diseases and pests, higher nutritive value (e.g., high content in oil, sugar, proteins, starch, vitamins), tolerance of some non-selective herbicides or stress factors such as extreme temperatures (hot weather or frost), drought, soil salinity and acidity.

The use of transgenic plants can result in (Bradford and Alston, 1990; Rosu, 1999):
- increased productivity, through efficient weed and pest control,
- larger profit for producers, due to lower production costs,
- an overall decrease in pesticide use,
- lower dependence on conventional pesticides that have negative effects on growers' and consumers' health,
- improved quality of surface and ground waters due to a reduction in pesticide waste.

The economic advantages of genetically modified plant use have led to a constant increase in GMO cultivated areas throughout the world. Since transgenic plants have been introduced on a large scale (1996), the cultivated surface has increased over forty-fold.

3. Regulation of GMOs

The overseeing of testing, use and commercialisation of GMOs — whether plants, animals or microorganisms — requires a special regulation system. This system establishes the legal and institutional framework for the control of potential negative effects of GMOs on the environment or human and animal health (Băbeanu, 2003).

In the US, transgenic plants are only introduced into the environment or on the market following approval from the following governmental agencies responsible for environmental, and human and animal health protection:

1. US Department for Agriculture (USDA),
2. Environmental Protection Agency (EPA),
3. Food and Drug Administration (FDA).

In the US and Canada, transgenic plants are grown and used for human and animal food, and separate storage and labelling are not mandatory.

Since 1990, in the European Union, special legislation has been drawn up, enhanced and extended, with the purpose of providing environmental and human health protection, and creating a common market in the field of biotechnology. Thus:

- EU Directive No. 220/1990, concerning the deliberate release of genetically modified organisms into the environment, was the main initiative taken by the EU, and was subsequently supplemented by several Commission Decisions (623, 811, 812, 813/2003),
existing regulations. This Directive also deals with mandatory information to the public, the long-term monitoring of effects, labelling and traceability, in all stages of GMO introduction on the market.

Two other acts have been adopted and published in the Official Journal of the European Communities, with respect to the Community system of GMO traceability, the labelling of genetically modified food and fodder, and the continuous procedure of authorisation or introduction of GMOs in the environment as food or fodder:

- EC Regulation No. 1829/2003 (22 September 2003) on genetically modified food and fodder, and
- EC Regulation No. 1830/2003 (22 September 2003) on the traceability and labelling of genetically modified organisms, and GMO-based food and fodder.


4. Ethical considerations

Biotechnology has expanded the tools available to geneticists and breeders, and the benefits derived from the application of transgenic plants may be applicable to human health and the environment, e.g. reduction in pesticide and fertilizer use, increase in plant production, etc. For developing countries in particular, the use of new genotypes (mutant and/or GMP) may be particularly important because modern agricultural technologies are not available for managing and protecting crops in these countries.

In fact, several authors (Bhojwani, 1990; Bradford and Alston, 1990; Gamborg, 2002) have cited the following positive aspects of GMP use in agriculture now and in the future:

- they are a valuable productivity aid, as plants with advantageous traits such as resistance and/or yield potential can be created,
- even more effort is being made to develop herbicide-resistant crop plants, some of which are already available,
- there was one product already on the market — the Flavr Savr tomato. This tomato has been genetically altered to manipulate the ripening process so that the fruit can be left on the vine for a longer period to improve its flavour. This product was withdrawn from the market for various reasons (high production costs, poor flavour, etc.),
- crop content is also a target for human and animal nutrition and the production of speciality chemicals, including biofuels,
- soil nitrogen availability is improved by (a) using inocula of bacteria which fix nitrogen from the atmosphere, (b) engineering improved efficiency in nitrogen fixation, (c) engineering crop plants to fix nitrogen,
- plants can be engineered to use light more efficiently,
- crops can be developed with tolerance to a range of environmental stresses, such as salinity, drought, frost and waterlogging,
- less artificial fertilizer and chemicals are used for crop protection, which reduces use of fossil fuels in agriculture, leading to considerable financial and environmental advantages.

Major reservations regarding the use of GMPs are as follows:
- the risk that new genes introduced in plants could escape and be transferred to other plant species in the ecosystem,
- some transgenic plants could promote new viruses,
- plant biotechnology may promote genetic erosion (i.e. a reduction in biodiversity),
- introduction of new transgenic plants could be an ecological danger to other species,
- insect-resistant transgenic plants could lead to insect resistance and destroy beneficial insects,
- herbicide-resistant transgenic plants could induce selection of non cultivated plants for resistance to herbicide,
- GMP development may lead to patent monopolies (patenting of genes and plants), which may in turn pose other moral problems.

In any case, as a biotechnological instrument for plant breeding, the PTC technique plays an irreplaceable and important role because it is used to:
- improve plant and seed health,
- conserve genetic resources,
- accelerate and disseminate genetic progress,
- increase the possibilities for creating varieties adapted to arid or difficult climates,
- increase the possibilities for creating varieties resistant to herbicides and pathogens,
- improve the quality of crops and foods,
- increase genetic diversity.

Recombinant DNA (or DNA manipulation) has huge potential for enhancing and extending the advantages of conventional plant breeding, and increasing crop production and productivity to meet the demands for food and food products in the future. Judicious application of this technology may alleviate some of the major constraints on crop productivity under subsistence farming conditions in developing countries.
However, biotechnology and, in particular GMPs, are no panacea for the many problems and challenges that the world faces at the dawn of the 21st century, such as health and environmental problems.

When the cultivation of genetically transformed *Zea mays* was authorised in France, the President of the *Institut National de Recherche Agronomique* said that when introducing an innovation, care is necessary and knowledge of the effects of introducing GMPs has to be evaluated. Moreover, he affirmed that as an alternative to immobility and irresponsibility, there is the reasonable position which requires the diligent evaluation of potential risks (Augée, 1995; Mizrahi, 1998).

The pressing questions about plant biotechnology are (Augée, 1995; Rosu, 1999):

1) whether the risks are properly evaluated; current EU regulations are inadequate because no notion of risk or methodology of evaluation are prescribed,

2) whether the consumption of food obtained from GMPs is dangerous for human and/or animal health. At present, this is not sufficiently clear, as conclusions from research are not available,

3) whether using GMPs can reduce the use of pesticides. Plant resistance to bacterial, fungal or animal attacks may result in parasites becoming resistant to toxins in transgenic plants. No scientific data is available on this subject.

Public debates on GMOs have gradually increased in some countries. Some of these debates have involved researchers, food producers, consumers and public groups, as well as decision-makers. Moreover, the public can be influenced by non-scientific considerations, but scientists, for their part, may be influenced by naive techno-scientific optimism; and some non-scientific considerations can be valuable elements in the debate about biotechnology. Scientists have no particular authority when it comes to judging moral issues.

Many people see a close connection between GMOs, food safety and the environment. Consumer concerns about GMOs mainly relate to food safety. Consumers are sometimes suspicious of the safety of food products obtained from new technologies. The problems raised recently by certain non-transgenic foods, e.g., allergens, pesticide residues, microbiological contamination and, above all, BSE, have increased these concerns.

Another public concern involves the fact that GMOs may cause ecological imbalances. GMOs are non-traditional products whose dissemination may result in modifications to ecosystem structure and functionality. These modifications may not necessarily correspond to the objectives sought. A risk of "genetic pollution" exists as a result of crossbreeding between GMOs and wild species.
The public is eager for information about GMOs used in agricultural production. Such information should be transparent and clear, and include details of the risks involved. Correct labelling of products, whether transgenic or not, is often mentioned as necessary for the protection of consumer rights. The public may want to actively participate in local, national and international debates, and express its opinions on the direction to be taken. But at present, the public needs to make efforts to understand the debates and make decisions on issues concerning GMOs.

The debate about biotechnology applied to agriculture is one of the most vocal and passionate debates to have taken place in recent years. This is probably due to the diverging beliefs that people and governments have of the real or potential risks and benefits that agricultural biotechnology products can bring. According to some, they would help to address some of the most serious problems that people, especially poor people in developing countries, face, such as starvation and malnutrition. Others argue that they could create serious and unpredictable health and environmental problems and have negative economic repercussions, in particular in developing countries (Bradford and Alston, 1990).

The proliferation of domestic biosafety schemes and the related authorisation, labelling, traceability, and documentation obligations are likely to further complicate international trade in genetically modified plants and/or agricultural products. For developing countries, agro-biotechnology is a particularly challenging phenomenon. They could be the main beneficiaries of it — if indeed agro-biotechnology keeps its promises — but they could also be the main losers if agro-biotechnology negatively effects biodiversity or if the patenting of biotechnological products and processes disrupts traditional practices among farmers and makes access to seeds more difficult.

Countries are free to decide how to deal with agro-biotechnology and biosafety at the national level, but domestic legislation has to be consistent insofar as it affects international trade. At the same time, this is a field where multilateral rules have been agreed in a separate legal instrument, the Cartagena Protocol on Biosafety (see www.biodiv.org/biosafety). The interaction between this specific instrument and international rules adds challenges to an already complex scenario.

While the developed countries have established their national frameworks for dealing with agro-biotechnology and biosafety by focusing primarily on domestic priorities and strategies, most developing countries are doing so under less flexible conditions. They increasingly seem to be expected to set up their national regulatory schemes based on the requests and expectations of their main trading partners. For developing countries, reconciling trade interests with their responsibility to improve the quantity and quality of agricultural and food products made available to the population and promote environmental preservation is proving to be a difficult task.
However, biosafety concerns have been raised regarding the use and release of GMPs. This debate has divided the farming and consumer communities over the acceptability of food derived from GMPs. There is a need for a thorough investigation of the effects of transgenic plants on the environment, and their interaction with wild relatives and non-target organisms. The production and release of transgenic plants should be based on experience and sound scientific reasoning. The regulatory requirements for the use of transgenic crops should be streamlined and harmonised, to achieve sustainable food production, poverty reduction and environmental protection in resource-poor countries.

Conscientious and responsible use of GMPs and/or derived products is needed to avoid mistakes that could lead to serious problems and increase consumer reticence. But plant biotechnology should not, and cannot, be discarded just because of a fear of the unknown. Today’s uncertainties should be overcome by a deepening of scientific knowledge as well as by continuous efforts to educate the general public and provide correct information about biotechnology. However sociological research has shown that giving the public more information about biotechnology does not necessarily lead to wider acceptance (Wynne, 2001).

Intelligent application of plant biotechnology may have much to contribute to agricultural and environmental sustainability while bringing value to producers, distributors and consumers. However, the commercialisation of such applications has been largely hindered to date, and additional research in both scientific and policy arenas is needed before opportunities for plant biotechnology can be expanded.

**Bibliography**


www.biodiv.org/biosafety
www.bioresurse.ro
www.fao.org
www.isaaa.org