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Plant Biotechnology in the Sustainable Agro-Food Sector: A Critical Overview

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Editorial

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When we talk about 'plant biotechnology' we should not immediately put the mind back to DNA and genetic engineering. In fact, since time immemorial, man has unknowingly used microorganisms to produce food and drink and has participated in the genetic modification of plants and animals through a gradual selection of the desired characters. The so called 'traditional biotechnologies' have allowed the production of beer, wine, bread or the transformation of milk into yogurt and cheese by exploiting the properties of prokaryotic microorganisms (bacteria) and eukaryotes (yeasts and molds). The awareness of the biological activity hidden behind these basic processes of the agri-food industry gradually began in the eighteenth century, with the introduction of the first optical microscopes, perfected thanks to the work of the Dutch optician and naturalist Anton van Leeuwenhoek (1632–1723). With Lazzaro Spallanzani and Louis Pasteur, the role of bacteria in fermentation processes and in the etiology of diseases was understood, laying the foundations for the industrial evolution of microbiology. A fundamental step in outlining the development of biotechnologies as we understand them today was the elaboration of Mendel's laws (1866) on the transmission of hereditary characters, with the consequent introduction of the terms 'genetics', 'genotype' and 'phenotype'.

With the description of the double helix structure of DNA given by Watson and Crick in 1953, molecular biology reaches an autonomous and complete definition, as a science that studies the structure and function of biological macromolecules, their transformations and metabolism relationships. In fact the work of Watson and Crick finally allowed to interpret and analyze the biological function and the molecular mechanisms carried out by the DNA: the self-replication, the transmission of heredity through the genetic code contained in it, and the regulation of cell development. The double helix structure has provided a model according to which DNA replication takes place thanks to a copying process of the single strands, while the action of the gene would be explained by a transcription mechanism in which the DNA acts as a mold or model for the constitution, through the activity of 'intermediaries' of the messenger RNAs, of the chains of amino acids that form the proteins. The novelty was represented by the discovery of a DNA molecule capable of transmitting information through a code, in which the sequence of nucleotides represents the encrypted message, interpreted by a system of translation of the base sequences into amino acid sequences. The specificity of the nucleic acid is entirely determined by the sequence of the bases, and this sequence is a code for the amino acid sequence of a given protein. The model of the double helix and the phenomenon of information transfer, offered the theoretical basis for the simple and universal solution of many questions, among others, the definition of the mechanisms of regulation of gene expression. Molecular biology, in particular studies conducted on enzymes that control DNA duplication and transcription (in particular DNA polymerases, which oversee the copying of a DNA strand, and restriction enzymes, which cut DNA at specific sites), then laid the foundations for the development, in the early 1970s, of the so called 'recombinant DNA technology', which allows us to isolate, analyze and eventually use known fragments of DNA of any species. From the development of this technique

genetic engineering has arisen between the 1970s and 1980s.

The concept of recombinant DNA from a scientific point of view is based on fairly simple criteria: identifying the gene of interest, cutting it and isolating it from the DNA molecule, joining the gene to a vector made up of DNA, and transferring it inside a receiving cell. The most widespread technology consists in inserting DNA fragments of different origin using small circular DNA vectors, the plasmids, deriving from bacterial cells of *Escherichia coli*. A wide range of different bacterial vectors and different *E. coli* strains are available on the market today for different needs in the cloning and recombinant DNA amplification phases. Cloning a DNA molecule therefore means producing a high number of copies of the DNA molecule being examined, an objective that can be *in vivo* obtained, through the use of bacterial vectors. An alternative procedure, which has greatly facilitated the work of molecular biologists, instead involves the amplification of DNA *in vitro*, through the so called 'chain reaction of DNA Polymerase' (PCR). The first strategy, using restriction enzymes and DNA ligases, allows to obtain recombinant DNA molecules, that is formed by segments of DNA of different origin, preserved in bacterial cells. The second strategy, by automating a cyclical process made of consecutive passages that are repeated many times, allows multiple copies of a DNA sequence to be obtained in a test tube.

The applications of biotechnologies concern different sectors, within which the fastest spread has taken place in the agri-food sector. The production of transgenic plants, that is the introduction of genes from other organisms into the genetic heritage of plants or the possibility of suppressing the expression of genes present in it, has revolutionized plant biology. The most efficient vector for exogenous DNA transfer in plants is represented by a bacterial plasmid contained in *Agrobacterium tumefaciens*, a bacterium of the soil that causes in nature a disease known as collar tumor (or gall) in its numerous plant hosts. What induces the tumor is, more precisely, a protein encoded by the DNA inserted in the Ti plasmid ('Tumor inducing') which consists of a circular double-stranded DNA molecule, of large dimensions and contains many genes implicated in the infectious process. The most relevant aspect of the Ti plasmid is that after infection a part of the molecule remains integrated in the chromosomal DNA of the plant cells; this segment is called T-DNA (or region T) and consists of that portion of nucleic acid, which carries the genes responsible for tumor growth, transferred within the chromosome of the host plant. It is therefore possible to construct vectors, derived from the Ti plasmid, in which the genes responsible for the appearance of the tumor are eliminated and in their place is inserted the exogenous gene that is to be transferred to the plant. In each transformation procedure, only a small percentage of the cells treated are actually transformed. In the preparation of a construct it is therefore necessary to use both selective dominant markers, such as resistance to an antibiotic, which allow the reproduction of only transformed cells when the corresponding antibiotic is added to the culture medium, both 'reporter' genes such as the Green Fluorescent Protein (GFP), a natural protein produced by the jellyfish *Aequorea victoria*, which when excited by radiation in the ultraviolet field emits green radiation (505 nm).

Biotechnologies are able to bring even very significant transformations in the agri-food industry. A classic example of the biotechnological development of a plant for food use is the so called 'Golden rice'. Golden rice is a genetically modified rice variety established in the 1990s by the Swiss Institute of Technology in order to enrich the rice seeds with β-carotene, a precursor of vitamin A. It was obtained by inserting in the rice genome two exogenous genes, psy and crt1, originating respectively from the narcissus and from the bacterium *Erwinia uredovora*. The purpose of this genetic modification is to give a positive response to the problems of malnutrition due to the lack of vitamin A in human population, a fundamental element for human health and whose diet is particularly poor in countries where rice is the main food. Despite the technological success of the project, and despite the announcement in 2005 of Golden Rice 2, which produces up to 23 times more β-carotene than the original Golden rice, this biotechnological solution has met with significant opposition from environmental activists and anti-globalization, which claim that there are sustainable, lasting and more efficient ways to solve vitamin A deficiency.

Not only can the expression of a new exogenous gene, but also the suppression of an existing gene determine an interesting genetic modification from the technological and industrial point of view. For instance, the 'gene silencing' is achieved through a mechanism based not on DNA, but on interference with the cellular processes of expression of the genes that are performed by the RNA. An example of this approach is given by the 'Arctic' apple, designed to block the action of the polyphenol oxidase (PPO) enzymes responsible for the browning of the apple pulp, which occurs immediately after the fruit has been cut. The reduced browning of the Arctic apple should favor the industry interested in fresh apple pulp products.

Genetically Modified Organisms (GMOs) are often phenotypically identical to equivalent traditional varieties, in the sense that the general appearance is no different, even if the GMO has acquired a new hereditary characteristic, due to the presence of an exogenous gene fragment or 'transgene'. Many concerns about GMOs have emerged regarding human health and environmental protection. The advantages deriving from the application of genetic engineering in the agro-food field are different:

- Resistance of crops to harmful insects (i.e. corn borer) or to pathogens (i.e. viruses, fungi and bacteria); tolerance to herbicides;
- Improvement of microbial species used in food processes;
- Creation of new products;
- Improvement of nutritional characteristics (as in the example of the Golden rice);
- Production of qualitatively improved 'tailored products' aimed at satisfying consumer needs. On the other hand, the main aspects of a risk assessment of the use of transgenic plants can be summarized as follows:
- The possibility that the exogenous genetic material is inadvertently transferred from GMOs to other non-GMO cultivated varieties, for example through cross-pollination;
- The possibility that the exogenous genetic material is inadvertently transferred from GMOs to other organisms, for example soil microorganisms through gene recombination mechanisms;
- The environmental consequences;
- The effects on human and animal health;
- Risk of monopoly by a few large producers of GMO seeds.

Nowadays, the legislation on the patenting of techniques, genes and GM varieties, and consequently the use of transgenic cultivated varieties and the trade of foods coming from GMOs, is absolutely not homogeneous in the various countries at the transnational level. Transgenic varieties are widely distributed in leading countries, such as USA, Brazil, Argentina, India, Canada and China. Corn, soy, cotton, canola, beetroot, alfalfa, papaya, pumpkin are the agricultural species with the largest areas occupied by GM varieties. In the US, for example, over 80% of the soybean grown belongs to GM varieties. On the other hand, the regulations in Europe on the use of GMOs in agriculture is extremely restrictive, and even for the future a change of this policy is not expected, substantially closed to the improvement of plants cultivated through the biotechnological approach.