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Rapport

Green biotechnologies: a strategic issue for the future of the French seed industry

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Abstract
Since man has domesticated plants and improved them, he has used every means at his disposal to do so. During the last 60 years, with the progress of knowledge in biology, especially in genetics, new tools, called "green biotechnologies", have appeared and are increasingly used. A working group of the French Academy of Agriculture has evaluated the use of green biotechnologies and identified their development potential by 2030 to meet the triple challenge of agriculture: coping with food security, respecting the environment and adapting to climate change. This report presents original information from a 2016 survey of 79 French plant breeding centres including 23 private companies and three public research centres of INRA (Institut National de la Recherche Agronomique; French National Institute for Agricultural Research), who were asked about the use of these tools.

Résumé
Depuis que l'homme a domestiqué les plantes et les a améliorées, il a utilisé tous les moyens à sa disposition pour le faire. Durant les 60 dernières années, avec le progrès des connaissances en biologie, notamment en génétique, de nouveaux outils, nommées « biotechnologies vertes », sont apparus et sont de plus en plus utilisés. Un groupe de travail de l'Académie d'Agriculture de France a évalué l'utilisation des biotechnologies vertes et identifié leur potentiel de développement à l'horizon 2030 pour répondre au triple défi de l'agriculture : faire face à la sécurité alimentaire, mieux respecter l'environnement et s'adapter aux changements climatiques. Ce rapport présente des informations originales issues d'une enquête menée en 2016 auprès de 79 centres français de sélection des plantes,

privés et publics de l'INRA, qui ont été interrogés sur l'utilisation de ces outils.

Keywords
green biotechnologies, breeding techniques, genome editing, seed market, France

Mots clés
biotechnologies vertes, techniques de sélection, réécriture du génome, marché semencier, France

Introduction

The Working Group on New Biotechnologies for Agriculture and Food of the French Academy of Agriculture addressed the question of the place of green biotechnologies in the French breeding of agricultural species. The Working Group made a qualitative and quantitative assessment of the biotechnologies used in plant breeding and specified the biotechnologies currently favoured by seed companies and their potential for development by 2030.
The study was built on the one hand from bibliographic works, consultation of databases and experts and on the other hand by interviews with managers of plant breeding institutes and companies present in France (76 centres of 23 companies of private research and three public research centres of INRA). The survey was performed in 2016. The objective was to gauge the current use of biotechnologies and their foreseeable evolution over the following 15 years.

Plant breeding has made a significant contribution to increasing agricultural yields in the 20th century

The genetic improvement of cultivated plants aims at developing new varieties that better meet the needs of users, farmers, consumers, industry and the new expectations of society. For this purpose, it seeks to accumulate traits of interest in varieties cultivated from the genetic resources present in the species (or related species) through breeding and crosses between plants that allow gene exchange. In the history of mankind, the improvement of plants started with the domestication of certain species in the Neolithic (ca. 10,000 BC) following the settlement of hunter-gatherers who become farmers, and which is at the base of the development of civilizations (Gallais, 2013).
Plants were chosen that had advantages for the quality of life (ease of harvest storage, etc.). In the course of the 19th and especially of the 20th century, scientific advances led to a rational approach to improvement, also called varietal breeding, which provided plants that were more productive, more resistant to pathogens and pests, and better qualities for the crop user (industrial users and consumers). These improvements were parallel to an improvement in cropping techniques, with an increase in inputs, including nitrogen fertilization. The result was a very significant increase in yields.
For example, since the 1970s, wheat yields have tripled with the Green Revolution (due to the introduction of dwarf genes in straw cereals); maize yields were multiplied by four and those of sugar beet by two. For each of these three species, a contribution of genetic improvement of 40 %, 70 % and 50 % is estimated, respectively (Gallais, 2015a).
Another example, the tomato, almost absent from the markets a century ago, become thanks to varietal innovation, the most popular vegetable consumed in France. There are many examples of improvement that have allowed vegetables to meet consumers' tastes (less bitter endives, green beans less friable, etc.).

France, the world's leading exporter of seeds and seedlings

Agricultural production relies on a strong seed sector that ensures the farmers' supply of quality seeds and seedlings. This channel also ensures varietal innovation that meets the needs of a globalized and segmented market in a rapidly changing economic and environmental context. This sector is experiencing significant growth (+ 65 % in the last ten years) with today a turnover of more than € 3 billion, half of which is exported. The positive balance of the sector's foreign trade was € 900 million in 2015, a third of the French agricultural balance. France is thus the world's leading exporter of seeds and seedlings. It is also the leading European seed producer and the third largest producer in the world, behind the United States and China (GNIS, 2017).

The importance of research in the success of the seed sector

Research is one of the success factors of this sector on the French market and for export. In fact, the lifespan of varieties is shortening and the turnover rate is very high: in France, the average age of the first ten varieties of the main species of field crops is less than seven years. In addition, the seed market is becoming increasingly segmented, so we need to create varieties that are adapted to each segment (GNIS, 2017).
Varietal innovations, and thus the research from which they come, are therefore essential for companies that wish to sustain their development or simply survive. Seed chain companies invest 13 % of their turnover in research, a proportion almost identical to that of pharmaceutical companies (14 %) and superior to that of electronics (9 %). The French private research budget has increased by 67 % in five years. It jumped from € 236 million to € 393 million in 2016 (GNIS, 2017).

In less than a century, green biotechnologies have upset varietal breeding

Green biotechnologies cover all *in vitro* and laboratory interventions on embryos, organs, tissues, cells or plant DNA, either to control or accelerate their production or to modify their characteristics.

Appendix 1 defines these different technologies. They result from a series of technological leaps that have taken place since the beginning of the twentieth century (Appendix 2), mainly: the re-discovery of the laws of genetics (in 1900), the modification of genomes (irradiation mutagenesis in 1928 by Stadler on barley), *in vitro* culture of plants (initiated by Haberlandt in 1902 and further developed by Gautheret in 1939), molecular biology (discovery of the DNA chemical structure in 1953), and in the twenty-first century, gene editing (in 2012, see Doudna and Charpentier, 2014).

Green biotechnologies: diversified uses. Green biotechnologies are very diverse and are used in breeding programmes for multiple purposes. There are three types of techniques that can be associated with each other: (i) *in vitro* culture techniques applied to immature embryo culture, protoplast fusion, micro-propagation, haplodiploidization, (ii) techniques that directly modify the DNA or genome sequence, such as mutagenesis, transgenesis or chromosomal doubling and (iii) molecular markers of the genome.
These techniques are used at various stages of improvement programmes to: exploit and expand the genetic diversity available to the breeder (embryo culture, gene transfer between different species, mutagenesis, gene editing ...); reproduce plants in the same way (micro-propagation); accelerate the duration of a varietal creation cycle (immature embryo culture, haplomethods, gene transfer); predict the value of candidate plants for breeding: marker-assisted selection, genomic selection.

A new era: precision genetics … New gene editing techniques, or site-directed mutagenesis, provide greater precision than random mutagenesis: we are now talking about precision genetics (Fichtner *et al*., 2014; Bortesi and Fischer, 2015). They make it possible to obtain new traits of interest related to resistance to diseases, to water stress, to nutritional or technological qualities, without affecting the rest of the genome. Thus, in the vineyard, site-directed mutagenesis would provide genetic resistance to late blight, contributing to greatly reduce fungicide treatments, without modifying the rest of the genome.

And quick response : Site-directed mutagenesis also saves time in creating varieties with the new traits obtained. Thus, currently by the conventional method of backcrossing, it takes about 25-30 years to introduce apple scab resistance. Using site-directed nuclease technique, the introduction could be carried out in five years and with a greater precision. These applications are expected to grow in the next 5-10 years. Proofs of the concept have already been made in many species: rice, wheat, maize, barley, sorghum, soybean, cabbage, tomato, potato, lettuce, orange, poplar, vineyard crops, etc. (Arora and Narula, 2017; Ricroch *et al*., 2017).

More effective, easier to implement and very economical, for the species where regeneration is controlled, these new techniques open up new prospects for the improvement of plants with the addition of new traits. They should allow for the improvement of orphan species (whose too small market does not allow the large investments required by other methods), and they will be accessible to both public and medium-sized private firms contributing to the limitation of the concentration of breeding companies.

On the other hand, the resulting plants seem identical to those that could be obtained by spontaneous mutagenesis, which is at the base of all the variation used by the breeders. The main problem remaining to solve is that plant transformation and regeneration is not controlled for all species (e.g., sugar beet, vineyard) or all genotypes of a crop species (e.g., corn) (Benson, 2000).

Taking into account all the advantages of these new techniques, it is therefore essential that they benefit from a non-discriminatory regulatory framework that allows their effective development in Europe. Conversely, discriminatory regulations would favour imports of seeds and agri-food products from third countries to the detriment of the French seed sector.

All types of agriculture benefit from green biotechnologies

Green biotechnologies make it possible to create, faster than with conventional methods, rustic varieties, resistant to diseases, and requiring fewer inputs for their enhancement. They are therefore also of interest for low input agriculture, including organic farming. This is already the case in wheat with the INRA variety Renan, widely used in organic farming, which results from interspecific hybridisation and embryo rescue techniques (Gallais, 2015b).

Promising prospects

The stakes for agriculture are rising to meet food security, agroecological transition and climate change. The French Agriculture Innovation 2025 plan has identified plant breeding and biotechnology as one of the major levers to respond to these challenges (Bournigal *et al*., 2015).

According to the Food and Agriculture Organization of the United Nations (FAO), biotechnologies are a real window of opportunity to help make agriculture more sustainable (FAO, 2016a). According to the Director of FAO "we need all the available tools, all the solutions, to meet the challenges of today, including biotechnology" (FAO, 2016b). In its new report on the prospects for science, technology and innovation, the Organisation for Economic Co-operation and Development (OECD) unveils the ten most promising emerging technology trends and focuses on four main strategic areas: digital, new technologies in the field of production (robotics, nanotechnologies, 3D printing), biotechnology and engineering of living tissue and, finally, energy and transport technologies (OECD, 2016). In France, the “Ecophyto Plan for Reducing the Use of Plant Protection Products”, in October 2015, has explicitly mentioned varietal improvement as a lever to achieve its goals (https://www.ecophytopro.fr/data/plan_ecophyto_2.pdf).

Biotechnology in the daily life of breeders: the results of the study

The companies and organizations surveyed in the current study (a total of 79 private and public research centres of 23 companies and three public research centres) have breeding improvement programmes on twenty-nine species or species groups: 16 field crops (bread wheat, durum wheat, barley, triticale, sorghum, corn, sunflower, rapeseed, sugar beet, potato, pea, lupinus, lucerne, faba bean, cocksfoot, and grasses for lawns) and 13 vegetable crops (cauliflower, garlic and shallot, carrot, bean, cucumber, zucchini, lettuce, melon, watermelon, onion, pepper, leek, and tomato). The field crops species that are concerned cover more than 14 million hectares, representing about 80 % of the total area in field crops, globally.
The current survey carried out made it possible to estimate the importance of different biotechnologies currently used in France in 2015 and prospects for 2030. Appendix 3 presents the biotechnologies used by species in 2015. Appendix 4 gives the relative importance of different biotechnologies used for the main field crop species. Appendix 5 lists the companies that responded to the current survey.
The main lessons of the survey are summarized below. Because of species-specific characteristics, not all methods are available for a given species. For example, *in vitro* regeneration is not yet available for all genotypes of maize, which could limit or complicate the implementation of site-directed mutagenesis in this species. The new techniques are adopted very quickly by the breeders and integrated into the routine of the research centres when there is no regulatory obstacle. By raising important technical brakes, they accelerate and increase the progress made by improved varieties.
It should be noted that random mutagenesis has been and is still widely used in many species. The mutations obtained have been integrated into breeders' genetic resources. Induced mutations have been used for 60 years and are present in 3,200 varieties in more than 200 cultivated species worldwide (https://mvd.iaea.org/), as well as countless varieties derived from these varieties. In this context, it is important to stress that events of induced mutations are largely used in different species, but very often the breeders do not use mutagenesis. They use induced mutants as natural mutants, ignoring their origin. For example, semi-dwarf genes are largely used in barley breeding, while they were already derived in 1965.
The three currently used techniques are molecular marker-assisted selection, which is carried out on a very large number of species, haplodiploidization and immature embryo culture. Green biotechnologies are widely used for the improvement of cultivated plants, field crops such as wheat, barley, maize, rapeseed, sunflower, sugar beet and the main vegetable species (Gallais, 2011, 2015a). They have been used for decades and have greatly contributed to genetic progress on different traits of interest to the farmer and the user (industrial and consumer).
Thus, today, except for “minor” species, almost all the new varieties that arrive on the market have benefited from one or more techniques derived from green biotechnologies. Over the next ten years, the contacted breeders anticipate a strong progression of genomic selection on the main crops and the progression of site-specific mutagenesis if it benefits from a suitable regulatory framework.

Seven Key Messages

1. Plant breeding has contributed significantly to the progress of agricultural yields in the 20th century. From the Neolithic era to present days, improvements of plants and of farming techniques are at the origin of major progress in agriculture, which guarantees an abundant, safe and accessible food/feed supply for the greatest number of human and farm animals. For example, in France, since 1970, yields have been multiplied by three for wheat, four for corn, and two for sugar beet. It is considered that varietal innovation has contributed to 40 % of gains in wheat yields, 70 % for maize and 50 % for sugar beet (Gallais, 2015a). Progress has made it possible to feed a world population in very rapid expansion: from 3 billion in 1960 to 7.3 billion in 2015.

2. The French seed sector: innovation at the heart of companies. This progress has been achieved thanks to the efficiency of our seed sector. This sector is made up of very diverse companies, small and medium-sized firms, cooperatives and subsidiaries of world groups, which benefit from various favourable factors for their development in France: pedoclimatic conditions, the know-how of farmers, a search for first order, notably through public-private partnerships, and an adapted regulatory framework.
This context has favoured the establishment and investment of private companies. On average, they invest about 13 % of their turnover in research and development, a percentage equivalent to that of the pharmaceutical industry. The French private research budget has increased by 67 % in the last five years. It jumped from € 236 million in 2011 to € 393 million in 2016 (GNIS, 2017). As a result, the positive foreign trade balance of the French seed sector reached € 900 million in 2015, a third of the French agricultural balance, once again putting France in the top position of global exporters.

3. Green biotechnologies for all type of agricultures. The methods and tools of plant breeding have been refined over time. In the beginning, plant breeding mainly involved mating systems (crossbreeding and selfing) and breeding. Advances in knowledge in biology, especially in genetics, have made it possible to develop a set of methods called "green biotechnologies". These include haplomethods, molecular markers, marker-assisted selection, genomic selection, site-directed mutagenesis, etc. They integrate into the breeders' "toolbox" to achieve four main objectives: exploit and expand genetic diversity, reproduce plants identically, accelerate the cycle of varietal creation and predict the value of candidate plants for breeding.

4. Green biotechnologies in the daily life of French breeders. New biotechnologies are rapidly adopted by breeders and integrated into the routine of research centres, as soon as they can be used technically and legally. The results of the current study show that the three main techniques currently used are molecular marker-assisted selection, haplodiploidization and immature embryo culture. The results of random mutagenesis have been and are still widely used in many species; mutagenic varieties have been around for almost 60 years.In 2015, FAO identified 3,200 directly mutagenized varieties, or mutagenic events, in more than 200 cultivated species worldwide (https://mvd.iaea.org/). Over the next ten years, breeders anticipate a sharp increase in genomic selection on the most important field-crop species (wheat, corn, sunflower, sugar beet and rapeseed) and the progression of site-specific mutagenesis, provided that it benefits from an appropriate regulatory framework in the European Union. Thus, today, except for “minor” crop species, almost all the new varieties that arrive on the market have benefited from one or more techniques derived from green biotechnologies.

5. A new era: precision genetics. Today, the latest techniques of site-directed mutagenesis (or "gene editing" or "rewriting the genome") provide greater precision and enable faster response to agricultural issues. They allow the development of traits of interest related to disease resistance, tolerance to water, and more generally abiotic, stress, nutritional qualities, etc. More effective, easier to implement and inexpensive, these techniques open up new prospects for plant breeding. Under certain conditions, they should also allow the improvement of “minor” or currently neglected species by varietal improvement programmes.

6. A necessary non-discriminatory framework in Europe. Plants derived from site-directed mutagenesis are indistinguishable from conventional plants. It is therefore essential that they benefit from a regulatory framework that enables their effective development in Europe. Conversely, discriminatory regulations would favour imports of seeds and agri-food products

from third countries to the detriment of the French seed sector.
It is not for the first time that governments struggle with regulatory challenges in the face of new technological developments. The current European Union genetically modified organism (GMO) regulatory framework was developed at the end of the 1980s following the development of recombinant DNA technology. This technology created novel possibilities to alter the genetic composition of crops and experience with the technology was still limited.
This prompted the European authorities to take a precautionary approach and consequently a regulatory framework was set up that required elaborate risk assessments and government authorization prior to the deliberate release and marketing of such GMOs (Custer, 2017). This was the birth of European Community Directive 90/220/EEC. In 2001, this Directive was replaced by EU Directive 2001/18/EC, which has an almost identical scope.

7. Green biotechnologies at the service of all types of agriculture. Contrary to popular belief, modern varieties from breeding companies' research are hardier, more resistant to disease, and require fewer inputs or value. They are therefore also adapted to low-input farming. More generally, the implementation of green biotechnologies benefits all types of agriculture. New biotechnologies can bring new traits of interest to all farmers while responding more quickly to their demands.
They should thus make it possible to respond to the triple challenge of agriculture: to face food security, to better respect the environment and to adapt to climate change. It is the mobilization of all the biotechnological tools and methods, and especially the site-directed mutagenesis, which will allow the maintenance of the strong position of France in the seed sector and thus the maintenance of a competitive agriculture.

Conclusion

Green biotechnologies have largely contributed to the improvement of plant species that feed the French population. Ongoing technological developments hold promise that in the next 10 years a wave of varietal innovations will help agriculture to meet the triple challenges of the 21st century: produce more, respect the environment and adapt to changing climate. They will concern not only field species but other species such as fruits, vegetables, vineyard crops, as well as minor or orphan species.
It is the mobilization of all biotechnological tools and methods and especially managed mutagenesis that will allow the maintenance of France's strong position in the international market for plant seeds, one of the few markets where we are positioned in the world's top three. Our agriculture will be able to remain competitive. It is therefore essential for these techniques to benefit from a regulatory framework that favours their efficient development.

Declaration of interests

Authors of this report are all members of the French Academy of Agriculture. This report does not commit the French Academy of Agriculture.

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Appendix 1: Glossary

Green biotechnology brings together a large number of methods and techniques that accompany the development and multiplication of cultivated plants. The main ones are listed below, in alphabetical order.

Genomics: field of genetics that studies the structure and functioning of genomes. It makes it possible to specify the position of genes on chromosomes, their function and the regulation of their expression. It relies heavily on DNA sequencing techniques and bioinformatics data processing.

Genotyping: constitution of genetic identity cards of plants (identification of certain areas of the genome, or even genes) so as to identify differences between them. This is one of the applications of genomics.

Genomic selection: a marker-assisted form of breeding that aims to accumulate over generations the maximum of favourable genes, even at low effects, thanks to a large number of molecular markers distributed throughout the genome.

Haplomethods or haplodiploidization: obtaining whole plants directly from the reproductive cells. The use of these methods reduces by at least half the time required for the creation of a variety that varies from 8 to 30 years. It is commonly used for the breeding of maize, rapeseed, rice, etc.

Hybridisation: controlled crossing between two types of plants belonging to the same species or to two different species.
In vitro culture: a method that makes it possible to multiply plants in large numbers. Thus, thanks to it, a single rose bud will for example provide 300,000 seedlings in a year compared with only 300 per plant with conventional methods. The cure of viral diseases of plants is also carried out by *in vitro* culture of meristems. In this way, strawberry plants were cured of the strawberry plant virus (Belkengren and Miller, 1962).

Marker-Assisted Selection (MAS): Breeding of plants using their genetic identity card, after establishing the relationship between molecular markers of DNA and the traits sought.

Mutation: a change in the DNA sequence that can result in substitution, addition or elimination of elements (nucleotides) of the DNA. This change can be punctual, a single nucleotide, or more important on a segment of DNA up to thousands, even millions of nucleotides. Mutations are at the root of the diversity of living beings, especially wild and cultivated plants (the domestication of plants began with the selection of mutations).

Random mutagenesis: all the methods that make it possible to increase the frequency of spontaneous mutations. For example, exposure to certain physical (e.g., ionizing radiations) or chemical treatments can increase this frequency by approximately 1,000. The breeder must then observe a reduced number of plants in the same proportion to identify and sort out the new interesting traits that have appeared randomly. Thousands of varieties grown in the world are derived from mutagenesis, such as sunflowers enriched in oleic acid, more colourful apple or grape varieties, seedless grapefruit, and so on.
Rescue of embryos by *in vitro* culture that would otherwise abort on the mother plant, especially in the case of interspecies crosses. This made it possible, for example, to obtain the triticale species, derived by crossing wheat and rye, widely grown today, particularly in organic farming, or the introduction of resistance to diseases in tomatoes from wild species.

Sequencing: determination of the sequence of elements (nucleotides A, T, G, C) of the DNA of a plant. The complete genome sequence of a corn, for example, includes about 2.5 billion nucleotides. It has been discovered that there is about the same number of genes in a plant as in a human: between 20,000 and 30,000 genes. More than 60 species among the most cultivated plants have been sequenced so far. Among which: apple tree (2010), banana (2012), bean (2014), blueberry (2014), cassava (2014), cocoa (2011), coffee (2014), clementine (2014), flax (2012), kiwi (2013), maize (2009), melon (2012), potato (2011), rice (2002), wild strawberry (2011), tomato (2012), vine (2007), etc.

Site-directed mutagenesis or gene editing: the use of recently discovered enzymes (nucleases) allows inducing at the genome level a cleavage of the DNA chain at a precise point; the mechanisms of DNA repair by gluing together the two parts of the chain will result in the loss of one or two nucleotides, which results in a point mutation. CRISPR-Cas9 or Cpf1 is the newest and easiest technique of its kind to use (Ricroch *et al*., 2017).
Somatic hybridisation by protoplast fusion: obtaining an entire plant from the *in vitro* fusion of two cells. This allows the creation of hybrid plants between species that do not cross.

Spontaneous mutation: mutation caused by different natural factors such as defects occurring during DNA copy, ultraviolet rays, environmental stresses and parasitic attacks. Their frequency is of the order of a mutation for 8 to 10 million nucleotides (see under "Sequencing" above) at each generation. Unknowingly, one harvests about 150 million mutations when harvesting one hectare of wheat.

Transgenesis: introduction into the genome of a plant of a DNA sequence containing a gene from a different species or not to bring a new desired trait. This gene is in general previously reconstructed in vitro to make it active in the plant. Genetically modified plants (GMOs) were grown on 185 million hectares worldwide in 2016 (ISAAA, 2016). Because of the regulations in Europe, companies do not develop such plants for the European market, except for Spain where the Bt (*Bacillus thuringiensis*) maize cultivars is allowed.

Method	First studies or applications	Interest	Some examples
Immature embryo culture	Hannig (1904)	Creation of interspecific hybrids (production of hybrids between plants that cross with difficulty although genetically close, ensuring the survival of embryos)	Tomato (the search for resistance genes to diseases is made from crosses with many species of tomato "Wild" such as *Solanum chilens* or *Solanum peruvianum)*
		Increase in the number of generations per unit of time. No need to wait for the formation of seeds	Sunflower (4 to 5 generations per year instead of one)
Random mutagenesis	Stadler (1927)	Irradiation of seeds (or vegetative organs) or use of chemicals to obtain new traits	Rapeseed (semi-dwarf rapeseed, modified fatty acid profile)
Chromosomal doubling	Blakeslee and Avery (1937)	Fertility restoration of interspecific crosses	Development of triticale
		Doubling of the chromosomal stock causing an increase in the size of the vegetative apparatus (leaf, stem, root)	Ryegrass (more suitable for grazing, more resistant to diseases)
Micropropagation	Gautheret (1939)	*In vitro* production of plants in the same way, in all seasons, in order to have a large number of plants without losing their properties. It also makes it possible to multiply plants that do not reproduce sexually	100% strawberry, leek (female parents of hybrid)
Meristem culture	Ball (1946)	Sanitation of cultures *in vitro* (in case of infection of plants by viruses)	100% potatoes (treatment of varieties infected with viruses from 1955)
Haplodiploidization	Blakeslee and Belling (1924)	Acceleration of breeding by obtaining homozygous lines in a single step (instead of 6-7 generations of self-fertilization starting from the crossing of two lines)	Rapeseed (by *in vitro* cultures of microspores), maize (by crossing with an inducer line), wheat (*in vitro* culture of anthers or crossing with corn or bulbous barley, with embryo rescue)

Protoplast fusion	Carlson *et al.* (1972)	Allows the exchange of cytoplasmic genetic material between related species	Cabbage, rapeseed, for the restoration of the chlorophyllian activity of male-sterile plants derived from crossing with the radish (Pelletier *et al.* 1983). Transfer of male sterility from sunflower to chicory
First use of molecular markers of the genome	Tanksley *et al.* (1982)	Study the genetic diversity of plants, genetic maps, gene tagging detection of QTL (Quantitative Trait Locus), etc.	Wheat, cabbage, rapeseed, corn, potatoes, etc.
Marker-assisted selection	Young and Tanksley (1989)	Use of molecular markers of the genome for introgression of genes, breeding of plants after crossing without phenotyping, accumulation in one genotype of favourable QTL with strong effects	Resistance to mildew in sunflower. Phoma resistance in rapeseed
Genomic selection	Meuwissen (2001)	Thanks to very high density tagging, breeding of traits under the control of many genes regardless of the importance of their effects	Tomato (fruit quality), corn

Species	Cultivated area in France (hectare)	Marker-assisted selection (genetics)	Immature embryo culture	Haplo-diploïdization	Meristem culture	Marker-assisted selection (biochemical)	Micro-propagation	Random mutagenesis	Protoplast fusion
Bread wheat	5 240 000								
Corn	3 300 000								
Barley	1 765 000								
Rapeseed	1 500 000								
Sunflower	680 000								
Sugar beet	435 000								
Triticale	390 000								
Durum wheat	320 000								
Pea	305 000								
Potato	170 000								
Sorghum	62 000								
Bean	32 000								
Melon	14 000								
Carrot	12 000								
Onion	11 000								
Lettuce	9 000								
Garlic and Shallot	5 000								
Leek	5 000								
Tomato	4 780								
Cauliflower	3620								
Zucchini	2950								
Cucumber	1 560								
Watermelon	886								
Chili pepper	595								
Species number		21	20	14	5	3	2	4	1

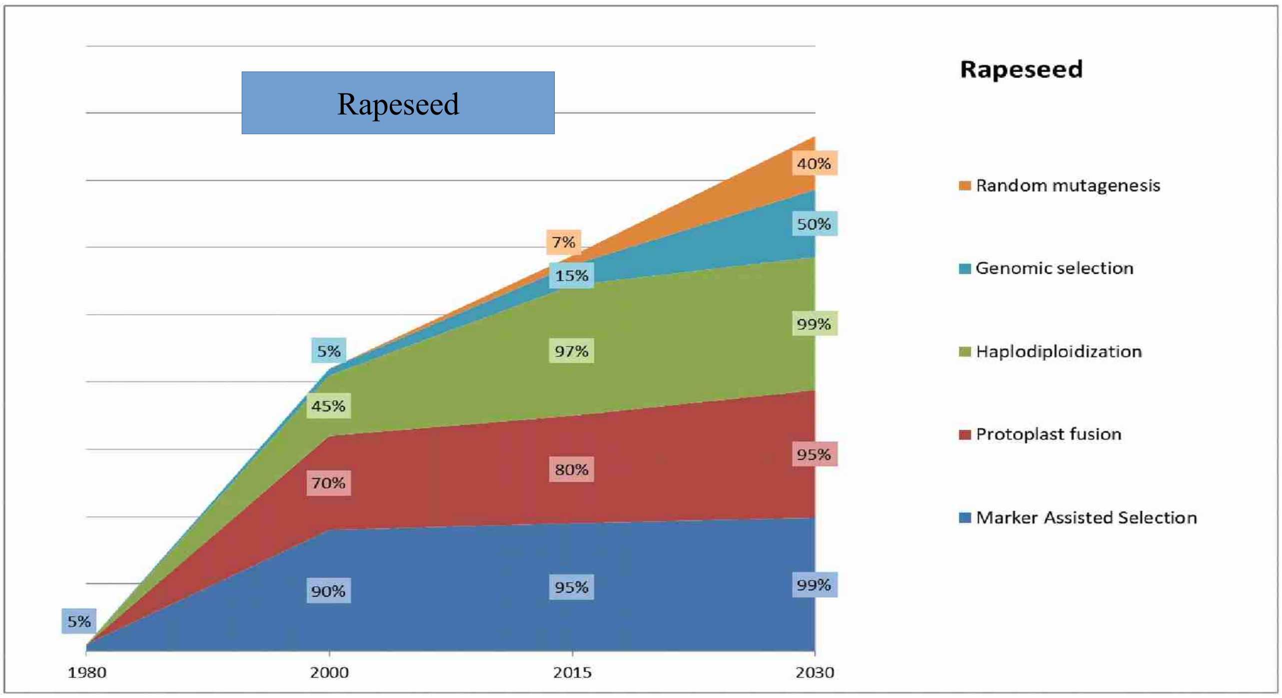
Appendix 4: Relative weight of different biotechnologies used for major cultivated species

This analysis was only possible for the species whose surveyed companies provide between 75 % and 95 % of the market and for which there is information on the market shares of the companies. The individual responses for 2015 and the 2030 projection were weighted by their effective market share in 2015. The results for the 1980s and 2000s are expert estimates. For this quantitative approach, only the main techniques cited by the companies have been exploited. Other techniques are used but more rarely, in the context of specific breeding programmes, or by only a few actors. The results are presented by crops of major agricultural importance.

The use of different biotechnological methods is presented in the form of graphs commented species by species (*see figures below*). The ordinate shows the percentage of the number of varieties of a crop on the French market that have benefited from a particular technique directly or via a distant relative (most varieties are the result of the cumulative use of several biotechnologies). Thus, for rapeseed, in 2015, cytoplasmic male sterility (derived by protoplast fusion) is used for 80 % of commercial varieties, haplodiploidization for 97 % of the varieties.

Rapeseed: Marker-assisted selection (MAS) developed dramatically between the 1980s and the early 2000s (Collard and Mackill, 2008). Biochemical markers were first used for levels of erucic acid and glucosinolates (undesirable molecules for food and feed), as well as for the lipid profile. The use of biochemical markers was only possible for certain traits. The development of molecular markers made it possible to have markers linked to all types of traits, for example for resistance to diseases (phoma, hernia) and the properties of the oil. By 2030, MAS should allow companies to advance on the protein content of cakes.

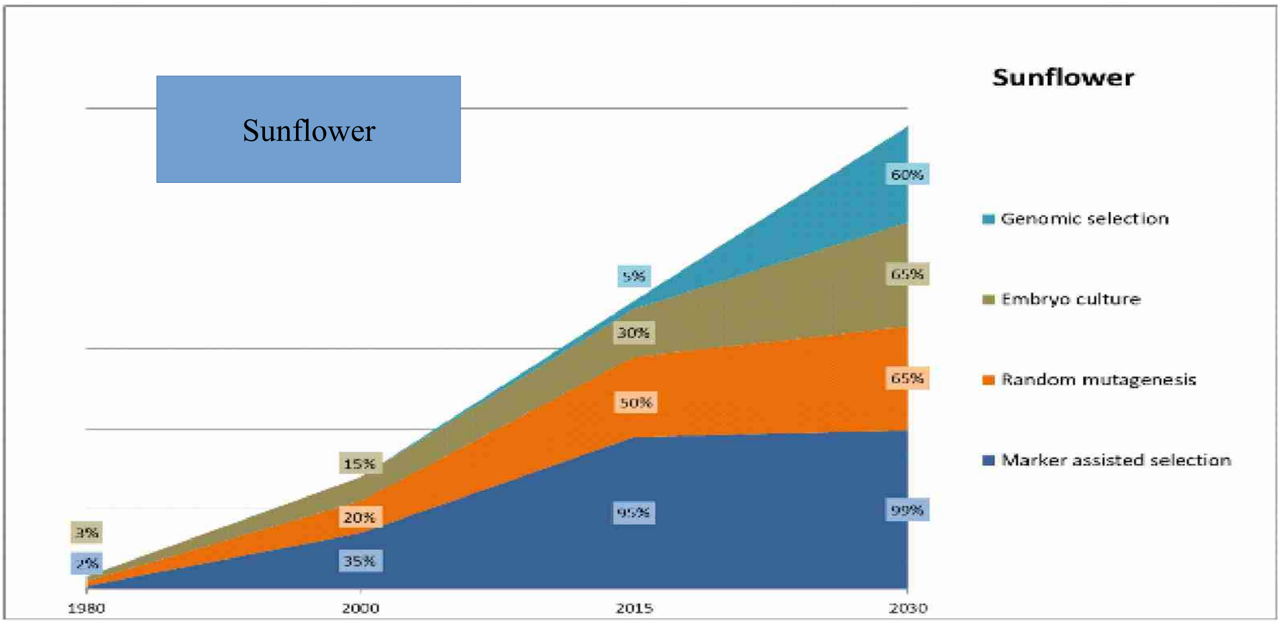
Protoplast fusion had a major impact for rapeseed with the development of the "OGU-INRA" cytoplasmic male sterility (CMS). In 1974, INRA carried out inter-generic crosses followed by immature embryo cultures *in vitro*, the first transfer of male sterility from radish to cabbage, then to rapeseed. The protoplast fusion is then used to restore the chlorophyll

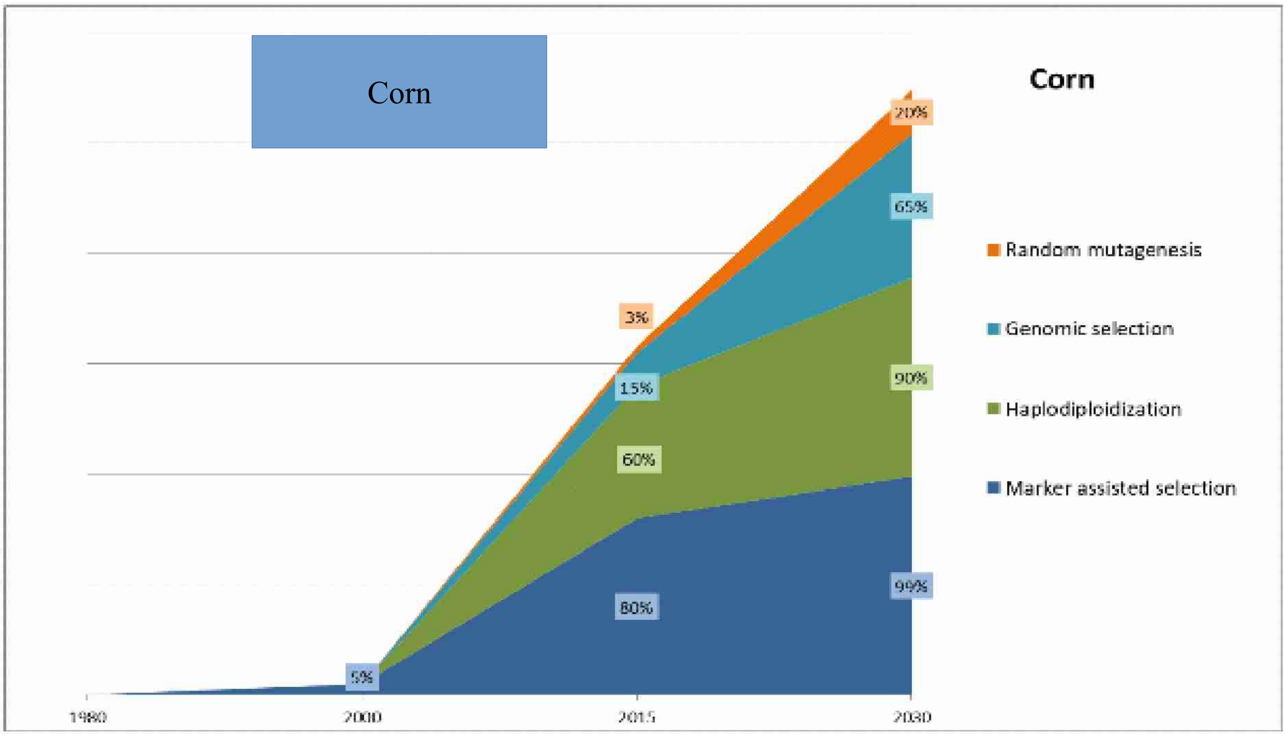


activity of the plants thus obtained. After several years of work, INRA patented the technique which is used for CMS in the production of rapeseed hybrids (Budar and Pelletier, 2001).
The haplomethods (by *in vitro* culture of micro-spores followed by spontaneous doubling) are very easy to implement for all rapeseed varieties and is very widely used.
Random mutagenesis via exposure to chemical agents or gamma rays has resulted in the creation of varieties with particular fatty acid profiles, herbicide tolerances and varieties with interesting cold-resistance traits for cultivation in northern Europe, thanks to INRA's isolation of the dwarf gene.
By 2030, this random mutagenesis should be replaced by site-directed mutagenesis (CRISPR / CAS 9 or CRISPR / Cpf1 tools) and allow the development of new traits of interest for rapeseed (Ricroch *et al.*, 2014). The projections of the survey in 2030 do not take into account a ban on their use that would result from the classification of these techniques in GMOs (the regulatory cost would be too high and the rejection of GMOs would bar the development of varieties that could possibly be marketed), but reflect the caution of the sector on the eve of decisive European decisions.

The culture of immature embryos is poorly used.

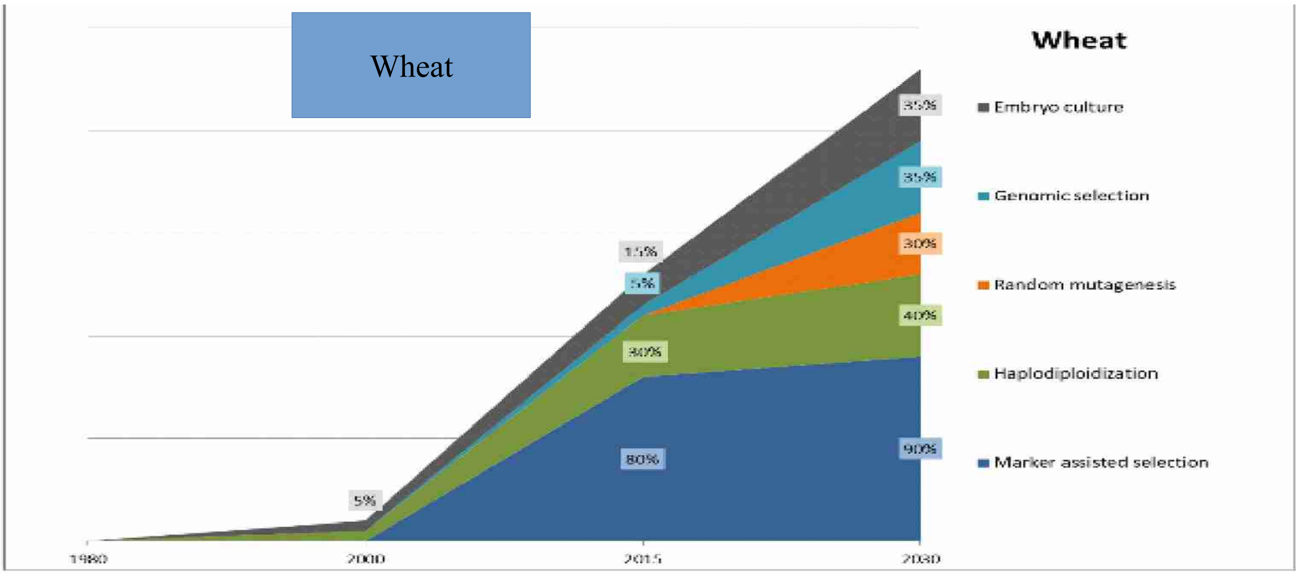
Sunflower (below): Marker-assisted selection is implemented in 2015 with molecular markers, used to "mark" resistance traits to diseases (mildew) and weeds (broomrape), as well as oleic acid and oil content. Currently, MAS is used routinely in the introduction of late blight resistance in commercial varieties.
Random mutagenesis (by chemical agents) has made it possible, thanks to a Russian programme developed in 1976 (Soldatov, 1976), to obtain varieties rich in oleic acid, which explains the important part of the varieties that have emerged since 2000. Resistance to herbicides was first discovered in wild sunflowers that had this characteristic in the USA, but subsequently this character was obtained through random mutagenesis. The development of this technique is hampered by the difficulty of regenerating whole plants obtained by *in vitro* culture for sunflower.
Embryo culture is very easy to use in sunflower and has been widely developed to accelerate the introduction of late blight resistance into new varieties.





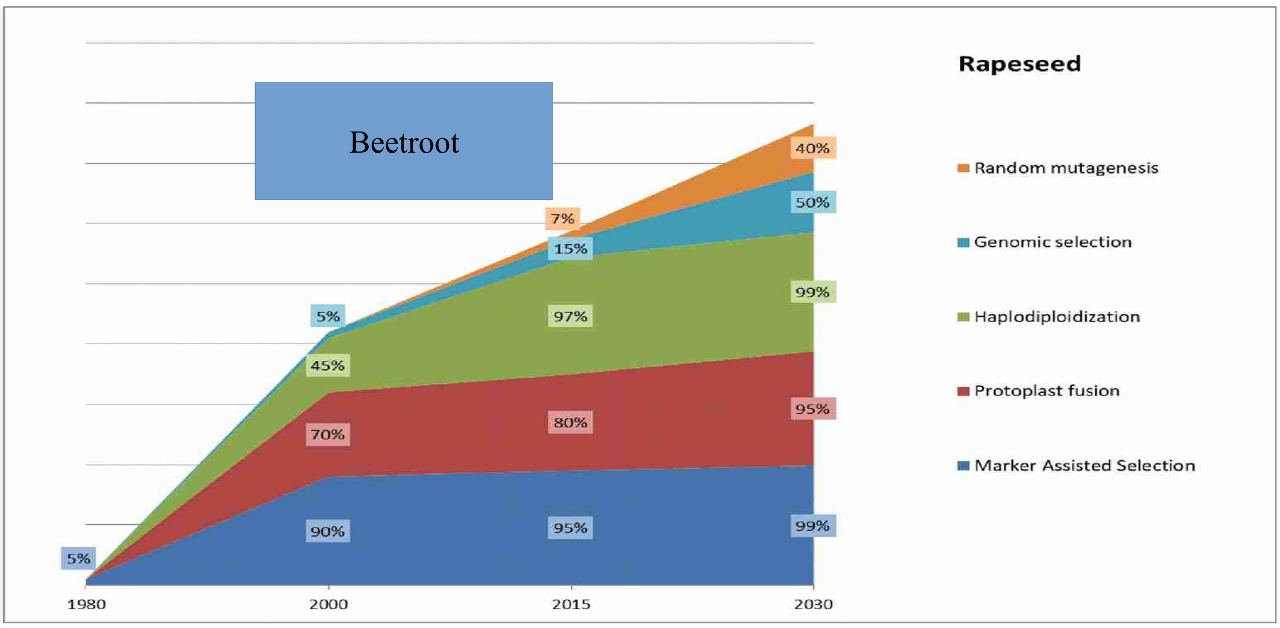
Corn (picture above): Molecular marker-assisted selection is widely used in 2015 with extremely rapid development in 15 years, due to the development of single nucleotide polymorphism (SNP) markers. It is thus used in routine breeding for the detection of many criteria such as resistance to diseases. Molecular markers are also used for the introduction of resistance, for the knowledge of genetic distances between parents, variability of lineages, ...
Haplodiploidization (by crossing with a genotype inducing haploidisation) allowed a clear acceleration of obtaining pure lines, necessary for the production of hybrids. It is widely used.
Immature embryo culture is sometimes used to accelerate generations of fixation.
Random mutagenesis represents only a very small percentage of the varieties on the market in 2015.
The use of site-directed mutagenesis would make it possible to develop new and interesting traits quickly, if the evolution of regulations so permits.
Genomic selection presents very important perspectives. It makes it possible to follow a number of important quantitative genes, which increases the precision of the breeding of the traits that are under the control of several genes (e.g., yield, earliness, corn silage digestibility). It is, however, little used for resistance to diseases.

Wheat: Marker-assisted selection and haplodiploidization techniques (anther cultures in vitro or pollination by bulb barley or maize) have developed significantly in the last 15 years (the first variety by haplodiploidization in 1983) and represent the principal techniques used with immature embryo culture (for interspecific crosses). The development



prospects of other techniques, such as genomic selection and site-directed mutagenesis, are real but will depend on their cost and the cost-effectiveness of their use for wheat species. Genomic selection could develop if there is a sharp drop in the costs of its implementation. The implementation of site-specific mutagenesis is dependent on the removal of regulatory barriers and technical difficulties.

Sugar beet: Chromosomal doubling, which was used until the 2000s for the creation of triploid varieties, is now very little used, with the development of diploid varieties. Marker-assisted selection used for the creation of all varieties on the market in 2015 and has experienced a rapid development for resistance to diseases. Genomic selection is under development.



Appendix 5: Public laboratories and private companies who participated in the current survey in France

Agri Obtentions
Barenbrug
Caussade Semences
DSV France
Euralis Semences
Florimond Desprez
HM Clause
Jouffray-Drillaud
KWS
Limagrain
Maïsadour Semences
Monsanto
OBS
RAGT Semences
SAATEN-UNION France
Sakata
Secobra
Syngenta
SESVanderHave
Soltis
Terre de lin
Unisigma
Vilmorin-MIKADO
INRA Laboratories (Angers, Avignon, Rennes)

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Rapport

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