OUR FUTURE:

GENETIC ENGINEERING?

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“The science of today is the technology of tomorrow”

-Edward Taller-

I would like to thank everyone who has supported me in this project from the beginning and that has collaborated with it in any way. Moreover, I would like to give special thanks to Dr. Fontanet from CRAG for attending me over the summer and providing me a bunch of information as well as their hospitality to visit their center. I am so thankful for all the support I have received from my family and finally I would like to give another special thanks to my tutor Marc for helping me with any kind of issue and for making everything much easier.

Abstract

The principal aim of this research project is to carry out a general analysis of the process GMOs (Genetically Modified Organisms) follow since they are created to the moment they get into the market and to see the effect these have on our society.

In the theoretical framework I will go through an explanation of this process whereas my practical framework will be consisted of three defined tasks; an analysis of GMOs in my own day meals, the realization of an experiment to extract the DNA (Deoxyribonucleic Acid) out of an organism and a brief explanation of my experience in CRAG (Center for Research in Agricultural Genomics).

I personally have always been so keen on everything that was related with science. It is because of this, and also to my curiosity to get to know more about the GMOs, that I decided to do my research project about them.

Compendi

El focus principal d'aquest treball és dur a terme una anàlisi general del procés que segueixen els OMG (Organismes Modificats Genèticament) des que són creats fins al moment en què entren al mercat, però també és conèixer l'impacte que aquests tenen en la nostra societat.

La part més teòrica del treball es basa en aquesta anàlisi, en canvi, la part pràctica està dividida en tres parts diferenciades; una anàlisi de OMG en els àpats d'un dia, la realització d'un experiment per tal d'aïllar el DNA (Àcid Desoxiribonucleic) d'un organisme i finalment, una breu explicació de la meva experiència al CRAG (Centre Recerca en Agrigenòmica).

El món de la ciència i els avenços tecnològics ha estat sempre un tema pel qual he mostrat força interès. Degut a això i també a la meva curiositat per conèixer tot el funcionament dels OMG, vaig decidir realitzar aquest projecte.

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1. Introduction

Ever since technology reached the food industry, GMOs have been one of the main topics for scientists to do some research on. By the time I heard about them I was in the US studying, my Biology teacher mentioned them in class and that really grabbed my attention. I immediately took an interest in these Genetically Modified Organisms because it was completely new for me and I had never heard about them. Moreover, I became aware of all the controversy these are currently creating and I awoke an interest and willingness to understand the because of this. I also wanted to learn the scientific view of the matter and to expand my knowledge about it.

For this, I tried to be objective and to contemplate all the perspectives of the topic. First of all, I was lucky enough to go to a specialized center in Barcelona and learn more about this magnificent world in the scientific area. On the other hand I got in touch with the *Departament d'Agricultura de la Generalitat de Catalunya* and they offered me the other point of view from the more social area.

As a way to explain my work in this project I decided to do an experiment, although my first thought of this was to carry out the whole process of making a GMO, I later became aware this was practically impossible without the appropriated installations and material. Therefore, I finally managed to synthesize just a part of the whole process and to extract the DNA out of an organism, which makes reference to a tiny part of the whole process of making a GMO. Moreover, I decided to prove if we actually do eat GMOs in our daily meals or not, for this I analyzed each and every single ingredient of my meals of a day.

In this research project you might find an answer to some questions about the GMOs. Going through an explanation of what they are, from the process they follow since they are created to all the polemic they generate, are they good or bad? The aim of this project is to expand my knowledge about the GMOs, of course, and to see how those are created; but also it is to inform people of what they really are and encourage them to develop a personal opinion about them through evidence and prove.

2. What are the GMOs?

By the time we are talking about the GMOs we are making reference to a living being. The symbol "GMO" is an acronym which stands for Genetically Modified Organism; it can apply to plants, animals, microorganisms and other living things whose genetic traits have been modified and altered.

The principal aim of this technique is to develop a new living thing with different characteristics so this can be more viable for whatever the desire is. It is important not to get the terms transgenic and GMOs mixed because just like all transgenic organisms can be GMOs, not all GMOs can be transgenic.

The word transgenic only encompasses those organisms that have been directly altered to the gene, those organisms whose DNA has been altered by the injection of another gene from a different organism. On the other hand, we use the word GMO for a more generic connotation, applying to all of those organisms that are genetically altered overall such as **transgenesis or gene delivery, cisgenesis, mutations, or grafting**. It should be pointed out that cloning is not considered a GMO since the DNA is being duplicated but not altered in any way.

2.1. Different types of GMOs

In order to avoid confusion when talking about the GMOs, I thought it would be a good idea to clarify the differences between some classifications of GMOs that should not get mixed.

2.1.1. Transgenesis or gene delivery

As it is mentioned before, transgenesis is consisted of the alteration of the DNA of an organism by the injection of some other gene from a different organism. This process tends to experiment and it also wants to improve or even solve some of the main challenges. For instance: with corn, some farmers would rather plant conventional corn hybrids[1] while other would go ahead and try the genetically modified corn product which makes sure some risks (like possible illnesses) and dangers for a normal corn plant disappear, making it more possible and profitable for farming.

[1] Hybrids: See glossary.

2.1.2. Cisgenesis

Cisgenesis is also called intragenesis and it might get confused with transgenesis; in fact, they are practically the same just with one difference. Unlike gene delivery, cisgenesis happens with genes from the same kingdom[2], same specie or even same gender. In cisgenesis, organisms must be sexually compatible in between them.

2.1.3. Mutations

Mutations are also considered genetically modified organisms since the DNA is being altered. These can be either voluntary (provoked) or involuntary (because of Mother Nature). However, it is important to differentiate between gene delivery and mutation. In a mutation the DNA is not being altered by the injection of a gene from a different organism but by the duplication, extraction or others (depending on the type of mutation) of a gene particularly from that same organism. For instance, many people think a watermelon without pips is a transgenic organism but the real true is that those are mutations. In this case, they are genetic copies of some chromosomes of the same organism (the watermelon).

2.1.4. Grafting

Grafting is a very old technique most farmers still use in order to cultivate their crops. It is consisted of growing two different plants together and so creating a new one by the joint of those. Hence, the final product is a genetically modified organism that farmers have been doing all throughout history without even realizing they were creating a GMO. On the contrary, it is thought by some people that cross-pollinating (grafting) is not a GMO since it is not being directly affected to the genome even though the final result is an offspring with a different set of genes.

3. How are the GMOs made?

Genetic engineering[3] has allowed researchers to go one step forward. As a result of which, organisms can be genetically manipulated according to the genes.

[2] Kingdom: See glossary.
[3] Genetic engineering: See glossary.

Every cell in our body contains over twenty thousand genes, which means three billion letters of DNA. DNA consists in two strands joined together by a double helix, adenine (A) pairs with thymine (T) and guanine (G) pairs with cytosine (C).

The process in which a GMO is made is pretty complex and it takes quite a long time to complete its totality. This process can be divided in two differentiated parts; the extraction and insertion of the DNA which happens in the molecular laboratory, and the growing phase that takes place in the greenhouse[4]. The injection of the foreign DNA happens as a result of Recombinant DNA (rDNA)[5], which brings the different genes together. Recombinant DNA differs from genetic recombination as this is a natural process; on the contrary rDNA is used in genetic engineering. Recombinant DNA uses restriction **enzymes**[6] which break down the chains of DNA to insert the desired gene, concluding the process with the addition of **ligase,** another enzyme that joins everything together. All of this, results in a final product made of DNA fragments (see **Figure 1**).

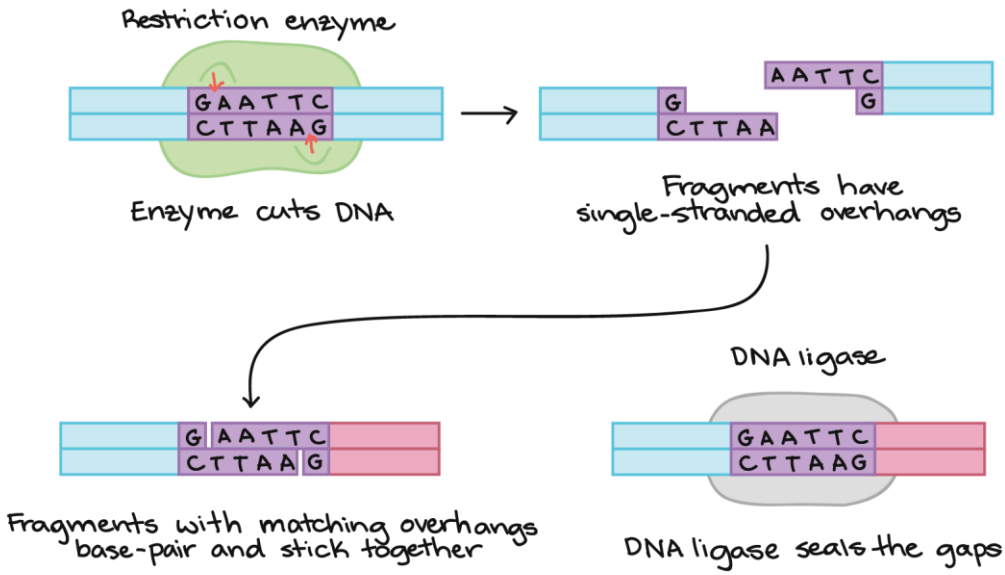


Figure 1. Diagram of how rDNA works, using restriction enzymes to separate the DNA chains and then joining them back together by using ligase.

Source: https://www.khanacademy.org/science/biology/biotech-dna-technology/dna-cloning-tutorial/a/restriction-enzymes-dna-ligase

[4] Greenhouse: See glossary.
[5] Recombinant DNA: See glossary.
[6] Enzymes: See glossary

Overall, both of these phases mentioned above (the molecular phase and the growing one) take approximately two months to complete its total process. However, the growing section of the organism might be longer as each living being behaves differently. Even though there is plenty of different ways to create a GMO (ones more applicable than others), the starting point is always going to be the same: figure out what the gene of interest is and afterwards, segregate it from the rest of the genes and insert it to the new organism. Here are some techniques used to carry out this molecular process of insertion of the foreign DNA by Recombinant DNA.

3.1. Electroporation

A really effective method to introduce foreign DNA into an organism is by using the technique of electroporation. However, this method is only valid for vegetal cells (monocotyledon[7] and dicotyledon[8]) as the first step to take is to get rid of the cell wall. In order to do so, **cellulose** (enzyme that decomposes the cell wall) needs to be injected into the cell. Then, once this treatment is in, it is going to form a **protoplast** which is another way to describe a cell without the cell wall, meaning that the cell membrane is exposed. Afterwards, as the name describes, electric pulses are going to be added to a suspension of protoplasts so it creates an electric field forming big enough holes (due to the permeability of the molecule) to introduce the new DNA through them. At the end, once this whole process is completed, the altered cell needs to stand for a while and let the cell wall form over again so it can be viable (see **Figure 2**). In this technique, though, the probability of cell death is really high. In fact, these conditions can cause approximately **40%** of the mortality of the cells.

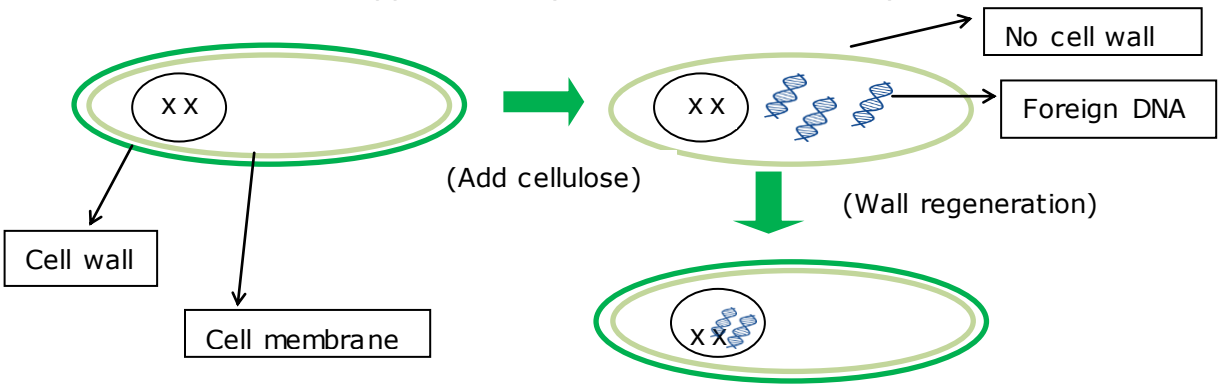


Figure 2. Graph showing the electroporation's process. Source: Own source.

[7] Monocotyledon: See glossary.
[8] Dycotyledon: See glossary.

3.2. Biolistic

This method, also known as *gene gun* or *bombardment transformation* is the most effective technique used to transfer DNA in GMOs. Biolistic allows DNA to be introduced directly into the cell, and provides more results as there is no cell death.

First of all, Tungsten[9] pellets are covered in DNA, these usually measure 1 mm of diameter. Immediately, the pellets are shot into the new cells using air pressure reaching a velocity of **400 m/s²**. Even though this technique might seem pretty simple, it is the most beneficial one for the cells and with more positive results. It can be applied to corn, rice, soy, wheat, and many others. However, it requires a bigger study after all since the number of cells that have been infected properly is unknown. Specific laboratory equipment is used to carry out this process (see **Figure 3**).

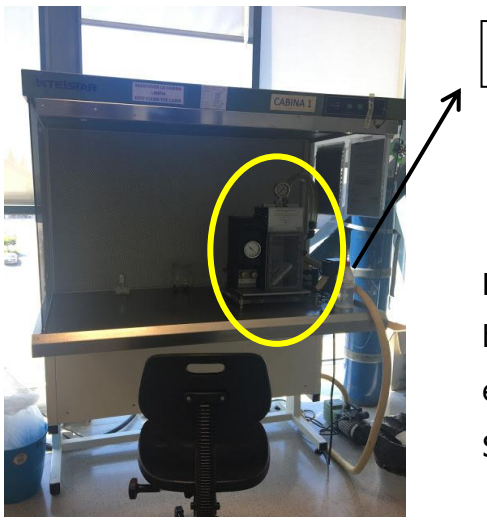


Figure 3. Laboratory equipment for biolistic. Cabin nº2, CRAG center equipment. Molecular laboratory.
Source: Own source.

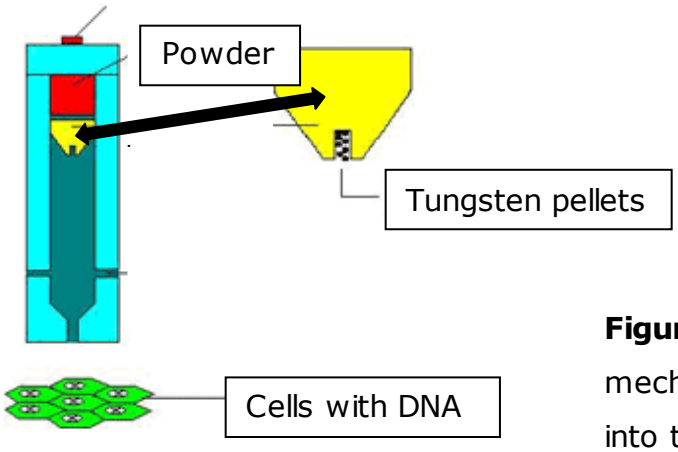


Figure 4. Graph showing the mechanism used to insert the DNA into the cells. Source: Own source.

[9] Tungsten: See glossary.

3.3. *Agrobacterium Tumefaciens*

Not all the methods have to be laboratory created; some of them can just happen naturally, such as the case of ***Agrobacterium Tumefaciens***.

This bacterium is found on the ground and its function is to take advantage of possible wounds of plants to get into the gene. *A.Tumefaciens* infects plants introducing foreign genes creating a tumor[10] or also called *crown gall*[11]. This tumor produces opines which is a derivative of amino acids[12], and it also contains a **Ti-plasmid** (tumor injected plasmid) which stores all the instructions to transfer DNA. A little portion of this Ti plasmid is the one going to stick into the infected cell, and it is known as the **T-DNA** (transferred DNA). Therefore, in genetic engineering it is possible to use this Ti plasmid as a vector[13] to inject foreign genes into a vegetal cell. Scientists inject the foreign DNA of the desired trait into this T-DNA section of the Ti-plasmid by using restriction enzymes and ligase[14]. Afterwards this synthetic plasmid of *Agrobacterium* is going to infect the new plant so it contains the new gene (see **Figure 5**).

This is a really effective method as it does not contain a really big genome and it only takes around a month and a half to complete its total process of injection. Nowadays, it is one of the most used methods for plant transformation.

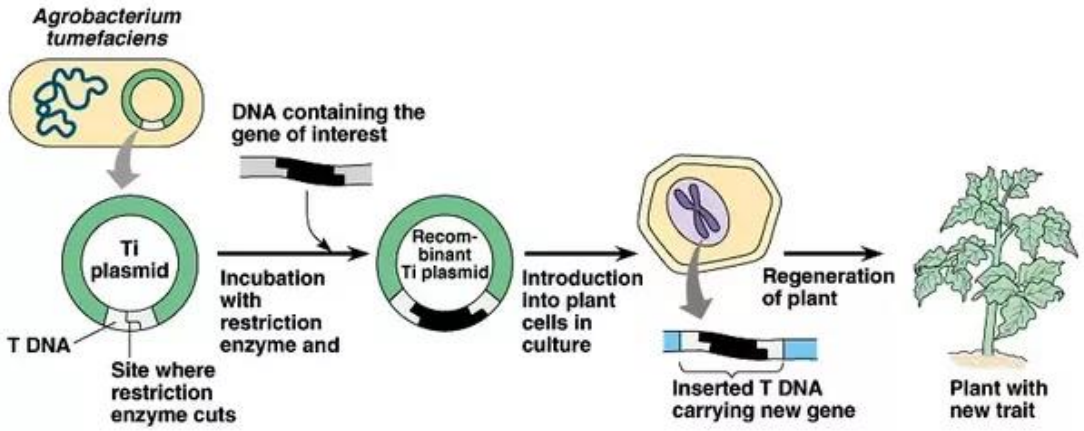


Figure 5. *A.Tumefaciens'* process. Extracting the Ti-plasmid from the bacteria to inject the desired trait into the T-DNA section of the plasmid for then insert this into the new plant. Source: https://www.quora.com/How-are-transgenic-plasmids-produced-using-Agrobacterium-tumefaciens

[10] Tumor: See glossary.
[11] Crown gall: See glossary.
[12] Amino acids: See glossary.
[13] Vector: See glossary.
[14] Restriction enzymes and ligase are explained before on page 11.

As mentioned before, *Agrobacterium Tumefaciens* is a bacterium that can infect organisms pretty easily. Therefore, the cleaning and the precaution of hygiene for this method must be really high so it does not infect the cells more than needed and kill them. In the following picture (see **Figure 6**), a cotyledon[15] is shown that has been infected by this. In the early process of cultivating the new cell, these can be infected either by bacteria, creating mucus, or by fungus[16] displaying a really different visual perspective.

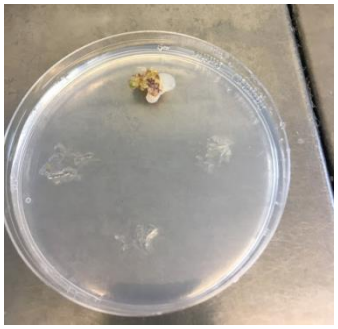


Figure 6. Cotyledon that has been infected by the bacterium *A.Tumefaciens.* Source: Own source.

3.4. Microinjection

In this process, scientists use micro needles to inject the foreign DNA into the desired and living cell. This tiny needle goes through the cell membrane and also the nuclear membrane to establish the DNA into the cells. Microinjections are carried out in a laboratory with special equipment and very precise machines that help the scientist do the work. This method requires a lot of precision and accuracy. However, it has some disadvantages; out of all the injected cells only a really low percentage end up getting infected and so workable. Moreover, this aleatory[17] injection might or might not alter other genes later. Microinjection is basically used in genetic engineering to do transgenic animals (see **Figure 7**).

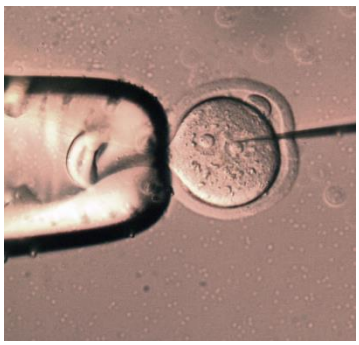


Figure 7. Figure showing how a needle injects the foreign DNA into a cell.
Source: Ph.D., Biomedical Sciences, State University of New York-Albany.

[15] Cotyledon: See glossary.
[16] Fungus: See glossary.
[17] Aleatory: See glossary.

3.5. PEG (Polyethylene Glycol-protoplast fusion)

PEG is a polyether compound with many applications, from chemistry to genetic engineering. Its name is an acronym for Polyethylene Glycol but it can also vary depending on its molecular weight. In order to do plant transformation, this is used as a stimulating compound for cell wall extraction. Alike electroporation, this method aims to create protoplasts by using cellulose. By **protoplasts** fusion, which refers to putting two exposed cells together, DNA can also be transferred. What PEG does in this situation is to act as a chemical compound so this process can be carried out (see **Figure 8**). The most used product for this technique is cereal.

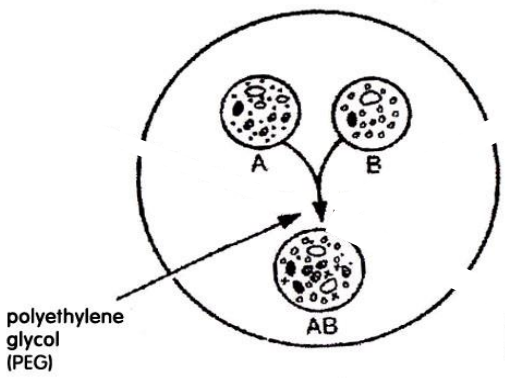


Figure 8. How protoplast fusion happens with the presence of PEG. Source: http://www.biotechnology4u.com/biotechnology_agriculture_methods_transfer_gen_plants.html

3.6. CRISR CAS9

CRISPR/Cas9 technology is a tool used in genetic engineering to modifying or correcting the genome of any cell. The initials CRISPR/CAS9 stand for Clustered Regularly Interspaced Short Palindromic Repeats; the second initial is the name of a sequence of proteins, mainly phosphodiesterase which were named as such because of the name CRISPR associated system.

Researchers have identified thousands of genes that affect our risk of disease and to understand how genes work, scientist need ways to control them. Recently a new method has been developed that affirms to dramatically improve the DNA of any specie. The CRISPR method is based on a natural system used to protect bacteria to protect themselves from infections by viruses. When the bacteria detect the presence of a virus, DNA produces two strands of short RNA, one of which contains a sequence matching that of the invading virus. These two strands form another structure with the protein called CAS9 which is a type of

enzyme that can cut DNA. Recently scientists have realized these can be used to cut any DNA in a really precise place. Afterwards the CAS9 will unzip the DNA and match to its target RNA. If this happens, the cell is going to start to repair the cut. In transgenesis a new sequence of letters is going to be added and replace the cut, forming then a new DNA with possibly different traits. Unlike previous methods, CRISPR can be used to target many genes at once and it can be used to study complex mutations that affect different genes at once, causing diseases.[18]

4. Types of transitions

Out of all the techniques explained above, there is none that provides a 100% of effectiveness for the DNA to stick in the cell. This is why all the cells need to be highly controlled after the molecular phase; to see if they actually got the gene or not.

In order to do this, scientists use what it is called a **selectable marker**; a substance that is added to the cultivation (before the plant starts growing) to see if the plant has the gene or not. In most cases it confers antibiotic resistance, so researchers can easily determine which cells have been successfully transformed. For instance, in *Agrobacterium Tumefaciens,* the selectable marker is this same bacterium that turns out being greener. Sometimes, it is impossible for the gene to stick within the cell. Hence, when this happens, it is known as a **transitory transition**. On the other hand, if results show the gene is still there, it is known as a **stable transition** where the gene is going to be stick into the nucleus meaning that the DNA is stable within the chromosomes.

Unlike mathematics, this is never a planned or expected result and the percentages of effectiveness of these methods are still quite low.

5. Uses of the GMOs

GMOs have revolutionized modern technology. The development of the agricultural technology is the best way to defy science and the world population growth. GMOs can go from being the solution to many illnesses to being a way

[18] See appendix 1 for further information about CRISR CAS9.

of overcoming the problem of children suffering from malnutrition. They are used in medical and biological research, experimental medicine and agriculture. Furthermore, these can be useful as a tool for many biologists in order to do some investigation in animals that are hard to study.

5.1. Medicine

GMOs in medicine have been a remarkable tool for progress such as obtaining new drugs whose synthesis is harder to obtain by doing it "in vitro"[19], creating new tissues and organs so the rejection risk is lower, or even fighting strange diseases such as Hepatitis B whose vaccine has been obtained from genetically modified yeast. All of these have allowed medicine to go one step forward and investigate. These are some ways GMOs have impacted medicine:

- Diabetes: In the past insulin (used to fight diabetes) was obtained from slaughtered pigs and caused allergic reactions. With the introduction of GMOs into the medical area, scientists created a new form of insulin made up of genetically modified bacteria (*E.coli*) which is healthy and safe for human body.
- Cancer: Genetically modified organisms are also a solution to several forms of cancer such as brain, colorectal, or cervical cancers. *Avastin bevacizumab* is a monoclonal antibody which fights against the growth hormone of the vascular endothelium (kind of tissue), and so braking the cancer.
- Anemia: People who suffer from Anemia mean that they void of transported oxygen within their bloods. *Epoetin alfa* is a kind of drug (made up of recombinant DNA) used to correct this disease which increases this.
- Vaccines: Some vaccines such as Cholera or Malaria are still under development for GMOs since non-GMO treatments are ineffective so far. On the contrary, some other strange diseases such as Hepatitis B have been fought with the incorporation of the exterior parts of Hepatitis B molecules into yeast cells.

[19] "in vitro": See glossary.

5.2. Agriculture

Plague resistance is one of the main reasons why GMOs are used in agriculture. Scientists create new organisms containing traits from another organism which give resistance to determined plagues that end killing the plant. Other organisms have been modified by the insertion of other genes that give resistance to weed-killers. Nevertheless, in general GMOs in agriculture are focused on the alimentary industry; to improve the elaboration process of some aliments such as bread or wine, and even to create a new and better version of a product to improve its nutritive qualities. Here are some examples of how genetically modified organisms are applied to agriculture.

- Corn: Corn is an example of a transgenic organism used in agriculture for plague resistance. For instance, European corn borer (*Ostrinia nubilalis*) destroys corn crops by burrowing into the stem, causing the plant to fall over. GM-corn avoids the presence of this animal providing then better production and a probable increase on the final cultivation.
- Soybean: Soybean is a crop whose cultivation is still not allowed in Europe, it is for that reason it needs to be exported from other countries outside of Europe. Soybean has many applications; from animal and human consume to the fabrication of paint and biodiesel. For this, genetically modified soybean possesses a trait that gives to the plant weed-killer resistance.
- Cotton: Its fluff has many uses in the paper industry and its seeds contain high quality oil (used to fry and as a compound for Margarine). Genetically modified cotton has been modified to show resistance to both, plagues and weed-killers.
- Potato: There currently exist two kinds of genetically modified potatoes; each one is modified for different reasons. ***Amflora*** potato was created in order to block a gene responsible of the production of amylose for then making it much beneficial for industrial production. On the other hand, the ***Innate*** potato was created with the following aims; to avoid the presence of bruises that detract from the product, to avoid its brownish color when exposed to oxygen, and to decrease its production of acrylamide when fried which is a compound suspected to be carcinogenic.

5.3. Bioremediation

Bioremediation is a scientific discipline based on the utilization of biologic agents, in a contaminated area or environment to degrade all of those components whose origin is organic and remove all of those whose origin is inorganic, by the process of chemical reactions. The start of the 80s supposed a grand revolution for the history of biotechnology and the first GMO was created, afterwards the study of the metabolic system for microorganisms allowed the design of the first genetically modified microorganisms (**GMM**). These microorganisms can be the alternative solution to creating wild strains that degrade slowly and do not contaminate at all.

It is important to look at the innovative perspective so the level of toxic organic compounds decreases and to maintain a better quality in the environment. For instance, oil spill is one of the main disasters that cause danger of extinction in animals and that also damage the environment. There exist GMM capable of repairing this disaster such as ***Pseudomonas spp*[20]*.***

5.4. Animals

Researchers have been genetically modifying mammals, birds, fish, and insects. While some of these are mainly used for medical research such as mice, others are modified to produce drugs or try to improve meat quality such as cows. There are some disagreements and concerns about introducing milk and meat from GM animals into the human diet.

Recently, the first commercialized GM fish has got to the markets. The fish variety of Atlantic salmon (*Salmo sala*r) is engineered to grow faster than its non-GM counterpart, reaching the market size in half the time an ordinary salmon would, which means that salmon actually reaches the market size in about 18 months instead of 3 years (non-GM-salmons).

This has been modified with the insertion of a gene that regulates the growth hormone of the salmon, which comes from the Pacific salmon (*Oncorhynchus tshawytscha*). And it also has been modified with the insertion of a biologic promoter called *Zoarces americanus,* to the 40.000 genes of the Atlantic salmon.

[20] *Pseudomonas spp:* See glossary.

All of these extra genes make salmon feed throughout the whole year and not just during spring.

6. Regulation of the GMOs in agriculture

There are differences in between the regulation of GMOs in different countries, some of the most marked differences occur between the U.S and Europe. Some people think genetically modified foods are prejudicial for our health while others see it as a great opportunity to develop new objectives.

Basing the facts on the survey I personally carried out, **65'8%** of the people who responded to my survey do not know whether these are prohibited to the market or not. In my opinion, I think society is misinformed. There is a lot of polemic existent with GM aliments for human consumption. Should GMOs be labeled? Well, the thing is different countries have different opinions about those in the world; the US, for instance, has been allowing their consumption in a really positive way since 1994. On the contrary, there are countries like France where they prohibited their consumption from the beginning. Nevertheless, each country has its own laws, and they need to be respected.

In short, how does each country regulate GMOs in agriculture? (See **Figure 9**).

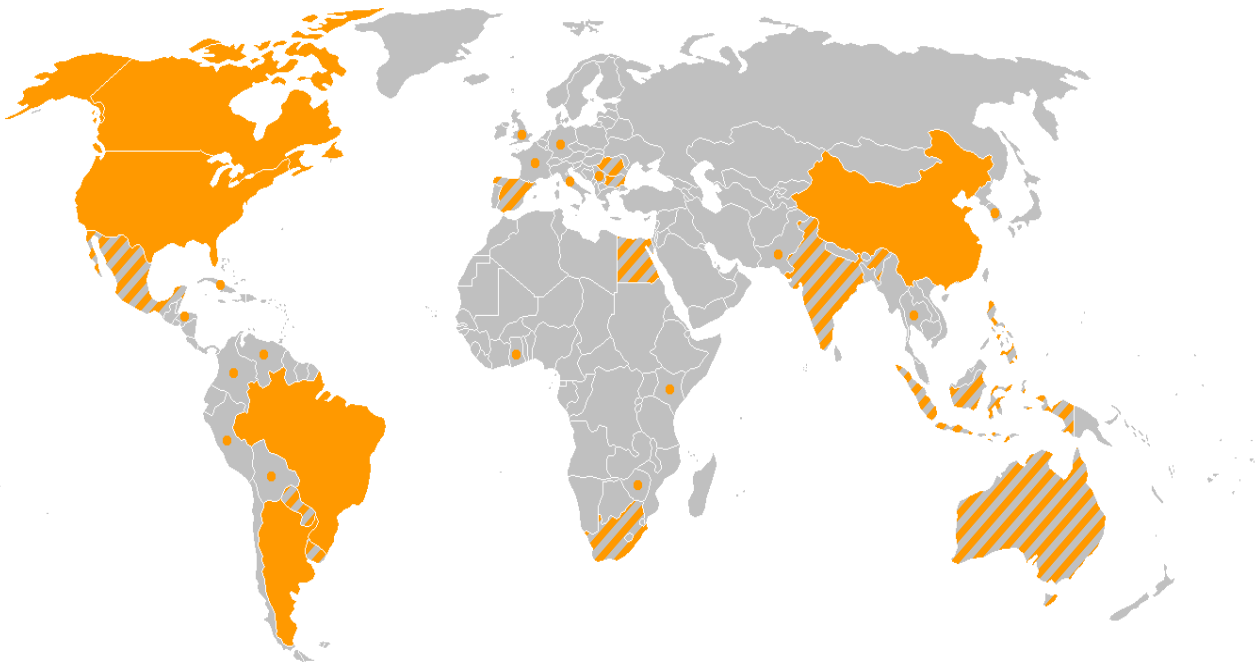


Figure 9. Map showing 2005 world's production of GMOs in agriculture.

Source: https://www.decodedscience.org/gmo-food-pro-and-con/23179

The map above is very significant as it shows in a visual way all the different countries around the world that grow genetically modified foods. There exist about 30 countries that promote this cultivation overall.[21]

In solid orange are countries that produce more than 95% of GM products. Orange and gray stripes represent countries that produce commercialized GM products. And orange dots represent countries participating in experimental GM crops. Gray means these countries have no relation regarding GMOs, neither production nor experimentation.

- Countries within this 95% of GM production: Canada, the U.S, Brazil, Argentina, and China.
- Countries that produce commercialized GM products: Mexico, Paraguay, Uruguay, Spain, Romania, Bulgaria, Egypt, South Africa, India, Indonesia, and Australia.
- Countries participating in experimental GM crops: Honduras, Cuba, Venezuela, Colombia, Peru, Bolivia, Ghana, Kenya, Zimbabwe, France, United Kingdom, Germany, Italy, Serbia, Pakistan, Thailand, and South Korea.

The worldwide surface of cultivated GMs reached 148 million hectares in 2010, which is equivalent to the whole territory of the United States of America.

At the present there are 27 GM crops all over the world which are: soybean, maize, rice, wheat, potato, tomato, beetroot, beans, endive, aubergine, pumpkin, papaya, watermelon, plum tree, sugar cane, alfalfa, Agrostis, cotton, flax, different varieties of rape, carnation, rose, petunia, sweet pepper, black poplar and tobacco.

6.1. The U.S

The regulation of the GMOs in agriculture in the U.S is divided in three different agencies: EPA (Environmental Protection Agency), FDA (Food and Drug Administration), and the U.S Department of Agriculture. These three companies regulate GMOs consume form different perspectives and make sure their

[21] It is important to remark that doing statistics is not easy when talking about something that keeps constantly changing.

consumption is steady and well-carried out. The U.S has been allowing their consumption since the start of the 90s and ever since.

Here is a timeline of how the U.S has evolved from the discovery of the DNA until now (see **Figure 10**).

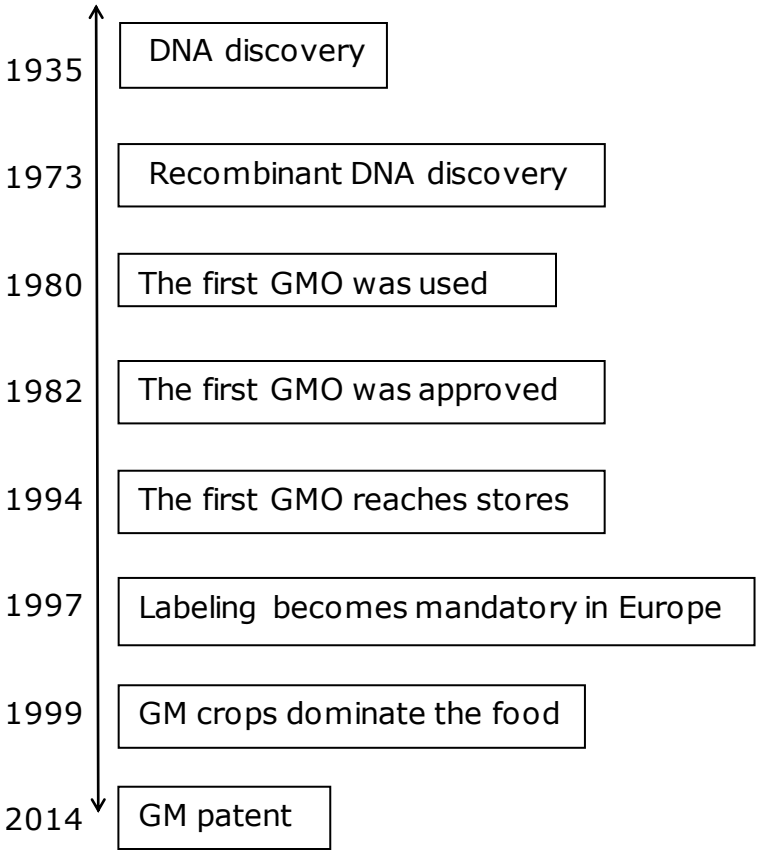


Figure 10. Timeline of the GMOs' history in the U.S.
Source: Own source.

According to the figure above the first GM crop in the U.S was created almost 40 years ago now and these have been out in the market since 1994. The timeline above shows how labeling became mandatory in Europe and how the U.S did not really pay attention to it. A lot of experimentation has been carried out ever since, and GMOs are still an entire world to discover.

As a new rule, food makers will soon be required to label their products when containing genetically modified foods although these labels are not going to be that obvious for the consumer. The proposed rule also instructs food makers to use the term *bioengineered* to label instead of using the term *genetically modified*, a more recognizable phrase.

The United States Department of Agriculture (USDA) declared food companies will have three options to label GMOs from now on; a one-sentence label declaration such as "contains bioengineered food ingredient", a standardized icon, or a QR that shoppers might visit for further information. USDA's plan will exempt refined oils such as those GM foods made from corn, soybeans and others. This huge number of exemptions already represents 70% of the food. Furthermore, the department will also exempt any kind of product containing less than **5%** of the genetically modified organism from labeling (it is 0'9 in Europe, Russia or China).

The United States now cultivates maize, soybean, cotton, canola, sugar beet, alfalfa, papaya, and squash taking up a total of hectares of 72.9 million, the number one in the world.

6.2. Europe

In Europe, before genetically modified foods are thrown into the market they need to go under some regulations. European Union needs to approve the product in terms of security to humans, animals, and the environment.

For this, genetically modified aliments follow a process:

- These are sent to EFSA (European Food Safety Authority) and within 6 months they must complete a form evaluating the product.
- After the form is completed, the product directly passes through European committee and it stays there for a period of 3 months long. There, they determine if the product is competent or not.
- Now, if the product is accepted, then it is ready to go to the market.
- Next, the accorded decision goes to the Permanent Committee of the Food chain and Animal health, consisted of all the representatives of each country in the European Union. There, it is judged and voted for absolute majority, which represents ⅔ of the tribunal.
- Finally, this is brought to the cabinet where this is voted again for absolute majority.

It is a complicated process and in case of disagreement the European committee is the one in charge of doing the ultimatum. In Europe, every GM product in the

commercial area must not go above the 0'9 percent of contamination without being labeled, distinguishable from the U.S which is 5%.

6.2.1. GM crops in Europe

At present, resistance against genetically modified products remains high. However, there are countries in Europe that keep producing and experimenting with the GMOs. Nowadays, **MON810** is the only permitted crop cultivated in Europe for non-experimental use. Out of all the countries constituting the European Union only the following 7 possess GM crops (see **Figure 11**).

	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Spain	53.667	75.148	79.269	76.057	76.575	97.326	116.307	136.962	131.538	107.749	129.081
Portugal	1.250	4.263	4.851	5.094	4.094	7.724	9.278	8.171	8.542	8.017	7.069
Czech	1.290	5.000	8.380	6.480	4.680	5.091	3.080	2.560	1.754	997	75
Romania		350	7.146	3.244	822	588	217	220	771	3	
Slovakia	30	900	1.900	875	1.248	761	189	100	411	104	138
Germany	950	2.685	3.173								
Poland	100	327	3.000	3.000	3.000	3.000					
TOTAL	57.287	88.673	107.719	94.750	91.193	114.490	129.071	148.013	143.016	116.870	136.363

Figure 11. Table that represents the amount of GM crops in the European Union in hectares. Source: Information from ISSAA and own source.

Out of all the biotech crops in the world (148 million hectares) Europe represented only its **0'09%** in 2016. The data above is relevant as it shows how Spain has increased its production over the years representing its **95%** of production within Europe. Portugal produced its maximum in 2013 and it has remained basically steady ever since. On the other hand, Czech Republic started reducing its production in 2012. Romania joined the production a bit later, in 2007 and it also ended in 2016. Slovakia also remains pretty steady whereas Germany brusquely stopped producing in 2009. In fact, same happened with Poland in 2012. France, in addition totally prohibited GMOs in 2007.

In short, Spain is basically providing the majority of Europe's production although it still represents so little quantity in terms of the whole world. In 2016

the total number of GM crops in Europe (highlighted in orange) was registered as an amount of 136.363.

At the present there actually exists a searching tool within Europe to find each and every desired GMO that is being accepted and commercialized in Europe, the name of which is Official Register of GMOs in the European Union.

6.3. Spain

In Spain, most part of the information from the European Union has been incorporated to Spain's legislation within the Law 9/2003, April 25th. This law promotes the confident use of the GMOs, the voluntary liberation and the commercialization of those. All of these, have been also incorporated to the Real Decree 178/2004 (from Spanish, Real Decreto), January 30th. This was modified and published known as the Real Decree 191/2004, March 15th.

In Spain, the Interministerial Council gives the needed authorizations to the National Commission of Biosecurity, which is an association body whose function is to inform and act as a consultative resource about the authorization solicitude of the GMOs. Nevertheless, if the rules are not followed properly the situation becomes complicated, and they might need to start talking about juridical terms such as penalties and fines. In this situation, there are three types of fines when the law is not obeyed.

- Mild fines: mild fines can get to the €6.000.
- Grave fines: grave fines go from the €6.001 to a maximum of €300.000.
- Very grave fined: Very grave fines go from the €300.001 to a maximum of 1.200.000€.

6.3.1. Labeling requirements

According to the labeling, in Spain, just as it is done all over Europe products must be indicated when containing a percentage above 0'9 of genetic modification. Otherwise, the law does not require labeling for those which do not go under these circumstances. Furthermore, in 2004 it was accorded to also label those aliments containing 0'5% when containing GM products with favorable risk but still not approved in the European Union.

6.3.2. GM crops in Spain

It is mentioned before[22] that Spain is a front country cultivating biotech crops producing **95%** of Europe's production. Some communities in Spain produce more than others and some do not produce at all such as *Euskadi*, *Asturias, Cantabria, Castilla y León* and the *Islas Canarias*. *Aragón,* produced 46.546 GM crops in 2016 placing itself on the top of the ranking for Spain, followed by *Catalunya* with a production of 42.567 crops.

At the present, as it is mentioned before there is only one permitted crop in Europe and so in Spain which is MON810 (MON, from Monsanto) and their derivatives (Bt-176), it is a kind of corn which is resistant to the plague of the **European corn borer**. However, 32% of the consumed corn in Spain is transgenic. This is the only crop whose cultivation is allowed for non-experimental use. Other than that, there are many other crops in Spain which are dedicated to experimental cultivations.

6.3.2.1. List of experimental crops in Spain

One of the main producers for biotech crops in Europe is Spain, but it also is one of the main countries which are more developed in experimental crops (see **Figure 12**).

Transgenic organism	Spanish Community	Investigation Aim
Adenovirus Ad5 CMV p53	*Catalunya, Galicia, Navarra, Madrid and Valencia*.	Antitumor genetic therapy
Poplar	*Andalucía*	Fast growing
Alfalfa	*Andalucía*	Tomato Mosaic Virus (TMV) resistance
Rice	*Catalunya*	Glufosinate-ammonium resistance
Zucchini	*Murcia*	Virus resistance
Plum tree	*Valencia*	PPV resistance
Rape	*Andalucía, Castilla y León*	Restoration of fertility
Eucalyptus	*Asturias*	Kanamycin resistance

[22] Mentioned before on point 6.2.1.

Transgenic organism	Spanish Community	Investigation Aim
Sunflower	*Andalucía*	Indicator genes and drought tolerance
Corn	*Andalucía, Aragón, Islas Canarias, Castilla la Mancha, Castilla y León, Catalunya, Extremadura, Galicia, Madrid, Navarra, La Rioja, Valencia.*	Virus resistance, insects, weed-killers, lepidopterist, masculine infertility, better nutritional quality.
Watermelon	*Andalucía, Valencia*	VMP, CMV, WWV-2, YMV and ZYMV resistance.
Potato	*Andalucía, Navarra, Euskadi*	Virus and fungi resistance.
Beetroot	*Andalucía, Castilla La Mancha, Castilla y León, La Rioja, Valencia*	Glufosinate-ammonium tolerance and resistance.
Soybean	*Andalucía, Extremadura*	Glyphosate and isoxazoles resistance.
Tomato	*Andalucía, Aragón, Castilla La Mancha, Extremadura, Murcia, Navarra, La Rioja, Catalunya*	Industrial and agronomic evolution, masculine infertility and indicators elimination.
Myxomatosis Virus	*Catalunya, Illes Balears*	Immune induction response in rabbits.

Figure 12. Table showing all the experimental crops in Spain.
Source: Information from Environment Ministry and own source.

As shown in the table above, many GM crops are cultivated in Spain for further research. The data above is very significant as it shows how every genetically modified product is used in science; for instance, Myxomatosis Virus is used in rabbits in order to promote their immune induction response. Spain is one of the few countries that promotes this research and donates it to science.

6.4. *Catalunya*

In the present, MON810 is the only crop whose cultivation is allowed in Europe; however there actually are 77 varieties hybrids from this crop that are cultivated

in *Catalunya* some examples of these varieties are: Bt11, NK603, MON863 x MON810 x NK603, MON89034 x MON88017, DAS 59122-7, NK603 x MON810, MON863 x MON810, MIR604 x GA21, DAS 1507 x NK603, T25, Bt11 x GA21, Bt11 X MIR604, DAS 1507, MON88017, MON88017 x MON810, Bt11 x MIR604 x GA21, GA21, MON89034, MON863 x NK603, MON810, 59122 x NK603, MON89034 x NK603, MON863, MIR604 or 1507 x 59122.

As far as an overall inform of the crops cultivated in *Catalunya* I found a study from 2012 that summarizes the number of cultivated crops of maize and it also shows the relation between non GM crops and the GM ones in hectares. In orange, I highlighted the quantity 24.001 which represents the number of hectares of GM crops cultivated in *Catalunya* according to the register in 2012. The table is the following and it is organized in provinces (See **Figure 13**).

Province	NO OMG (ha)	OMG (ha)	TOTAL (ha)
Barcelona	1.661,62	56,15	1.717,77
Girona	4.719,18	5.662,58	10.381,76
Lleida	12.366,79	18.257,52	30.624,31
Tarragona	20,67	24,69	45,36
CATALUNYA	18.768	24.001	42.769

Figure 13. Register from 2012 showing the cultivated surface of maize crops in Catalunya. Source: Information from "Department d'Agricultura de la Generalitat de Catalunya" and own source.

7. Controversy

The topic of genetically modified organisms is one of the most strongly debated subjects around the world today. Despite all the benefits for science as well as human beings GMOs might provide, they can also generate a lot of polemic. Most scientists and other people see genetic modified aliments as a way to discovering new things by investigating and improving aspects of human nature; while other people such activists and ecologists see this as a threat to their health. GMOs are the basis of alimentation in the US and other countries

whereas they are basically prohibited in Europe. Here are the different perspectives of the matter put in an objective way.

7.1. Supporting GMOs and their benefits

A huge percentage of people suffer from hunger in the world. By using modern plant breeding methods, such as GMOs, this could get to an end. There is a lack of knowledge and information about genetically modified organisms. For instance, it is thought that nourishing from an animal that has received some genetically modified food is dangerous and can affect whoever is feeding from that. But the real truth (referring to scientific terms) is that even if the animal has received any kind of GMO or not, acids and other kind of gastric substances within the stomach break down each and every protein chains. There's nothing wrong with having a mix of genes from foreign organisms in our genomes.

As the Genetic Literacy Project quotes "we share 24 percent of DNA sequences with wine grapes, 44 percent from honey bees, and 73 percent from zebrafish"[23]. The only thing that makes a gene human or plant is the fact that the humans share genes with other humans and plants do the same with other plants. Many people are misinformed about GMOs and think they can be prejudicial when they are just basing their hypothesis on false facts. Taking some results of the survey I personally carried out into account, I can see how the **52%** of the people who responded to my random survey think they are prejudicial for human consumption.

GMOs have been scientifically tested by government agencies in the US and declared as safe as non-GMO is, both for consumption and for the environment. An expert in nutrition like Abel Mariné, professor at the Faculty of Nutrition in the UB affirms that GMOs are not prejudicial for neither human nor animal health at all. Perhaps allergies could show off after a period of time of consumption since a new product is being injected to the organism. But allergies are always going to be present, with or without the existence of GMOs. Professor Mariné, during an interview in *Els Matins de TV3*, compares the fact of using the scientific method in the gene delivery to the one in the artificial insemination.

[23] *The Genetic Literacy Project,"* Genes we share"

Both of the situations need to be tested for further results, but they also need to be given an opportunity.

GMOs can help improve nutrition properties of aliments aimed to human consumption. Illnesses like HIV could be abolished with further investigation; malnutrition could also be prevented with new aliments rich in proteins and carbohydrates; and so on.[24]

7.1.1. Golden rice fights blindness

Third world countries do not have at their disposal the same resources other countries do. Things that seem to be as basic as the abundance of Vitamin A turn into a nightmare when it is deficient. Blindness has become one of the main problems in some developing countries. Every year, around 2 million children die or suffer from this. In the 90s, scientists became aware of the abundance of rice these countries had at their disposal, so they started to look for a solution to the problem. With the entrance of the GMOs in science, some realized that by increasing its B-carotene, the precursor of Vitamin A in rice, blindness could be treated. After doing a lot of research, in December 2017, this was approved as a food source and nowadays people of Bangladesh (the country in which this was first tested) are taking advantage of this Golden Rice that it is actually saving children's life.

7.1.2. Global movements supporting GMOs

The principal world-wide promoters of the GMOs are **Monsanto and Novartis.** They are the ones who control the mercantile business at the moment. Most of the commercialized products such as corn, cotton and tomatoes come from Monsanto. This company is working to help farmers grow food more sustainably.

7.2. Against GMOs and their inconvenient

On the other hand, many people think GMOs have become a huge negative impact in human life. Not even just for consumption but for the environment and the agriculture. For the environment, it is thought genetically modified seeds, from the organisms that have been already altered, travel well beyond fields where they are grown, contaminating the surrounding crops. According to the

[24] See an example on section 7.1.1.

concurrent legislation, GMOs can be sowed with special permissions with the requirement that they need to be at least 25 meters away from other regular crops. Otherwise, there is high risk of infection through pollen transport.

For human consumption and fauna, scientists say GMOs have reduced the 10% of the butterflies' populations or impacted animals in some other ways and that by the time we realize it is going to be too late. It is said GMOs increase antibiotics resistance making them less effective for human health and increasing cancer or other illnesses possibilities.

Furthermore, for agriculture, many people who are against GMOs affirm grand companies benefit from that leading farmers and retail marketing down. They say genetically modified organisms are just one of the many ways they take advantage from in order to make money. GMOs provide a better version of the cultivation, making the most of land's output since these are resistant to many pests and diseases. This derives a lot of benefit from selling these seeds to farmers worldwide. Another point to take into account is the natural flavor. People against GMOs complain about food's taste, they argument GMOs do not taste the same as a natural product since it has been genetically altered.

7.2.1. Global movements against GMOs

***Greenpeace**, Amigos de la Tierra or Ecologistas en Acción* are some global movements that are currently playing a really big role in trying to avoid this commercial product. *Greenpeace*, though, strongly affirms they are prejudicial for human health and that they must not be consumed. After doing some research, *Greenpeace* has evaluated the following results.

- **3%** of world's aliments are made up of GMOs.
- There are currently **17** countries in Europe where GMOs are prohibited.
- **67%** of the investigations in crop fields are carried out in Spain (within Europe)
- **99%** of the farmers are still indecisive in cultivating biotech crops.
- **61%** of the population affirms GMOs must be labeled in all commercial products.

Greenpeace also made a list of some genetically modified food that is being currently commercialized in Europe.

7.2.1.1. *Greenpeace's* list

As it is mentioned previously, besides corn GMOs are not allowed to be commercialized in Spain. However, if the percentage of genetic modification is below 0'9, they can be commercialized since it is not even declared a GMO. Hence, *Greenpeace* as a way of protesting decided to make a list (with all kinds of international brands) of these eatable products that are currently available in the market. These products do not need to be labeled because of the minimum percentage of modification they have although some of them are.

The following list attached to this research project, is not the full version but a reduced one of it (see **Figure 14**).

Kind of product	Brand
Chocolate	*Toblerone* *Kit Kat* *Twix* *Nestlé* *Crunch* *Nesquik* *Cadbury*
Ketchup	*Heinz*
Mayonnaise	*Hellman*
Fizzy drinks	*Minute Maid* *Pepsi*
Chips	*Lay's* *Pringles* *Cheetos* *Ruffles*

Kind of product	Brand
Oil	*Carbonell* *Koipe* *Fenómeno* *La Masía*
Butter	*Flora* *Ligeresa* *Tulipán*
Canned food	*La Cocinera* *Calvé* *Miggi* *Solís* *Knorr*
Drinks	*Nesquik* *Nescafé* *Ricore*

Figure 14. *Greenpeace's* red and green list.

Source: https://archivoes.greenpeace.org/espana/Global/espana/2015/Report/transgenicos/GuiaRojaVerdeTransgenicos_5edicion_Actualizacion062015.pdf

8. Eating GMOs on a regular basis

In order to verify all the points mentioned above about the human consumption of the GMOs, I decided to put my knowledge one step forward and prove whether the products we all eat in a regular basis contain even a tiny bit of

genetic modification or not. For this, I took into account Greenpeace's list and each and every labeling, noted down my meals in a day, and investigated each and every of the aliments put on my plate that day. The outcome resulted to look like this:

8.1. My meals in one day

Breakfast:

- Milk, *Llet Nostra*. No, milk has not been genetically modified.
- Cereals*, Chocapic*. Yes, *Nestlé*'s chocolate has been genetically modified even though it is not expressed in the labeling.
- Cookies, *Digestive*. No, digestive is an ecologic brand that does not genetically modify their products.
- *Nutella*. No, this kind of chocolate has not been genetically modified.

Lunch:

- Noodles, *Gallo*. No, *Gallo* does not modify their products.
- Green and red pepper. No, coming from the garden at home.
- Onions. No, coming from the garden at home.
- Shrimp. No, the labeling affirms not containing GMOs.
- Squid. No, same as the shrimp.
- Salt. No, salt has not been genetically modified.
- Fried tomato, *Solís*. Yes, Greenpeace's list affirms being genetically modified.
- Oil. Yes, almost all oils have been genetically modified.
- Fish broth, *Aneto*. No, the labeling affirms not containing GMOs.
- Watermelon. Yes, watermelon has been genetically modified to extract its pips.

Dinner:

- Chicken. No, chicken has not been genetically modified.
- Oil. Yes, almost all oils have been genetically modified.
- Mustard. Yes, Mustard has been genetically modified.
- Honey. No, honey has not been genetically altered.
- Zucchini. No, coming from the garden.
- Onion. No, coming from the garden.

- Aubergine: No, coming from the garden.
- Salt. No, salt has not been genetically modified.
- Banana, Cavendish. No, this type of banana is not currently available as a GM food.

In conclusion, out of all the aliments inserted in my day, just the 2'6% had been genetically modified meaning that here, in Europe most of them are prohibited for human consumption.

By the time I was analyzing each ingredient I realized most of them did not contain any kind label, meaning the percentage of genetic modification was not above 0'9. Therefore, it is very hard to be aware of what it is being eaten by just looking at the product. In order to fully understand whether a product is a GM or not, research into every product needs to be done.

9. How to extract DNA out of an organism?

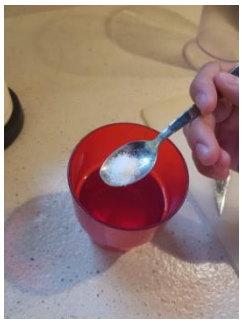
Since the practical framework of this project tends to be pretty poor due to the lack of equipment to carry out an experiment, I decided to look for something that had a relation with the main topic, GMOs. Therefore, I became aware of an experiment that could be carried out at home: extracting DNA out of an organism.

The ability of extracting DNA is something most scientists do in their regular basis. For instance, finding out whether organisms have any interesting mutations in their genes which can make it worse or better, and so on. Learning how to isolate DNA has become the starting point for many applications in biology and genetic engineering. In order to make a GMO, the first step to take is to extract the DNA off the organism which is being modified. Thus, within the practical framework of this project I decided to describe, in a very simple and casual way, how to extract this DNA out of an organism. In this case, I actually used a banana as the organism. It is a pretty simple process that can be carried out at home without any kind of special equipment. Here is how you get the DNA out of a banana (see **Figures 15-28**):

The first step is to peel the banana and chop it in really small pieces so the solution afterwards is a homogeneous mixture. For this, I used just half of the

banana but this can change depending on the amount of DNA willing to be extracted.

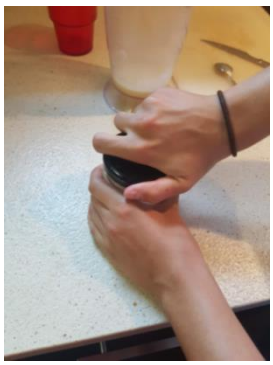
Then, a teaspoon of salt is added to half a cup of warm water and those are dissolved together in a blender. It is important to get the solution mixed homogeneously.



Next, once the mixture is uniformly mixed, the solution is poured into another recipient. In addition, about a teaspoon of detergent needs to be added to that solution, more or less. Now, the point of this is to break the plasma membrane in which the DNA of each of the cells of the banana is wrapped in. So then, I stirred the mixture for about five minutes or so.

Once these five minutes were completed, I saw the plasma membranes of the banana cells had been broken down by the detergent, releasing some DNA inside. However, this was still not visible since that DNA is still mixed up with other elements inside the cell such as lysosomes ribosomes, carbohydrates and much more. If I had happened to be in a laboratory, I would have centrifuged

the mixture spinning it around separating the heavier part from the lighter ones. But, since I did not have such at my disposal, I decided to take advantage of a stocking and put the container in this (it is important to check its lid is covered) and spin it around for about half a minute.



Once the centrifuging task is done, the sample left should be divided in two differentiated parts. Most of the denser components of the cell had gathered down the bottom while the less dense ones, including the DNA were floating around in the middle. The result after the centrifugation process looked like the following picture.

Afterwards, I poured that a bit further with a strainer until the point I did not have any chunks of banana remaining. At this point the DNA was dissolved but I wanted to make it visible with the naked eye, so I added alcohol really carefully forming a nice layer on top of the sample. The reaction the alcohol generated in contact to the sample made it visible as I continued precipitating it.


A white and cloudy substance started to form, that turned out to be the DNA, showed in the picture below (see **Figure 28**)

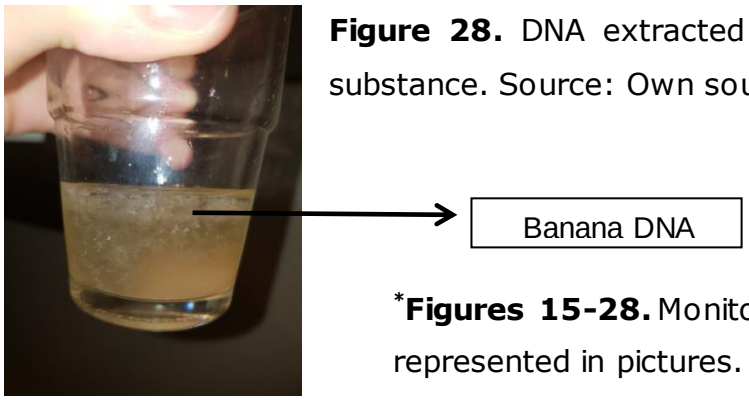


Figure 28. DNA extracted off the banana visible in the substance. Source: Own source.

*__Figures 15-28.__ Monitoring of the experiment represented in pictures. Source: Own source.

By the result of this experiment I deduced the following parameters.

In the picture above, DNA appears to be shown as a bubbly chain. It is visible with the naked eye and it does not dissolve. The DNA material becomes visible almost immediately when the alcohol is poured into the banana. The DNA strands are visible when grouped together but too small to be seen with the naked eye when talking about the individual strands. However, the DNA seems to be fragile and deteriorated quickly. This is the base of all genetically modified organisms, without the DNA extraction, organisms could not be modified.

10. CRAG installations

As soon as I started working on my research project I decided to get myself well-informed. This is why I contacted with CRAG, a center in Barcelona which put me in touch with Dr. Pilar Fontanet, who is a Biologist working in this same center in the section of plant growing facilities.

CRAG is a center devoted to leading-edge research in the molecular basis of genetic characters of interest in plants and farm animals and in the applications of molecular approaches for breeding of species important for agriculture and food production. Research at CRAG spans from basic science to applied studies in close collaboration with industry. The building of CRAG is located in the UAB campus in Bellaterra, Barcelona (see **Figure 29**).

Dr. Pilar Fontanet was the one who welcomed me to the center and there my experience consisted of two days.

Figure 29. CRAG's building in Bellaterra and Dr. Pilar Fontanet and me.
Source: Own source.

The first day I visited CRAG, on Wednesday July 4th, I was showed all the installations around needed in order to create a GMO, they introduced me what was done there and, I was informed of many aspects of the GMOs. There I saw all the special equipment needed to grow a GMO; from the beginning in the molecular laboratory (see **Figure 30**), to the green houses (see **Figure 31**).

Figure 30. Molecular laboratory room in CRAG.
Source: Own source.

Figure 31. Greenhouse in CRAG.
Source: Own source.

Growing plants in process were barely planted, so they proposed me to visit their installations once again when these were already a little more grown up.
On July the 13th, I was called to visit CRAG again and there they showed me the molecular laboratories and the process a GMO follows until it is in the greenhouse, which is the following:

After the foreign DNA is introduced into the desired trait in the molecular laboratory, the little culture containing the cell goes to the acclimatized chamber so it can grow faster and with more effectiveness since it is more controlled. There are two kinds of chambers showed below; the closed little chambers (see **Figure 32**) and the walk-in ones (see **Figure 33**).

Figure 32. Closed chamber in CRAG. Chamber number 1. Source: Own source.

Figure 33. Walk-in chamber in CRAG. Acclimatized at 24 Celsius degrees. Source: Own source.

After many checking steps making sure that the cotyledon does not contain contaminated areas caused by neither bacteria nor fungus, and the cell has become bigger and it does not fit in the Petri dish; it goes to the greenhouse. There, the plant first remains with a plastic wrap so the acclimatization in the greenhouse is not that much of a brutal change in the environment for the plant. Then, after approximately a week, scientists start to make some holes in it (see **Figure 34**) and finally to retire it and let it grow till the point scientists can do their conclusions and finish the previously started project.

Figure 34. Acclimatization process of a plant in the Greenhouse in CRAG.
Source: Own source.

(I also had the opportunity of seeing some plants such as watermelon, tomato or tobacco).

Finally, there I was lucky enough to get introduced to an intern, Miguel Ezquerro who was doing his thesis for his doctorate. They both, Dr. Fontanet and Mr. Ezquerro facilitated me some information of what they were doing there and I had the opportunity of having at my disposal the tomato protocol they were using at that moment to cultivate transgenic tomatoes[25].

In genetic modification, not all the alterations to the genes express a phenotype[26], some just remain the same outwardly and some do not. According to this protocol, though, it does show a difference between the non-genetically modified tomato and the one which is not (See **Figures 35-36**).

Figures 35-36. The photos above show the difference between a non-genetically modified tomato plant on the left, and a genetically modified one on the right. These tomatoes have been genetically modified applying to the explained protocol above. They are in the greenhouse phase.
Source: Own source.

The tomato that has been genetically modified appears to be purplish and actually bigger whereas the one that has not been genetically modified remains red and not as big. This is an example of experimental design applied to science which shows an **independent variable**[27] which is the altered gene, a **dependent variable**[28] which is the tomato plant and many **constants**[29] such as the temperatures and the lightning. All of these results in a well carried out experiment.

[25] The whole tomato protocol appears on Appendix 3.
[26] Phenotype: See glossary.
[27] Independent variable: See glossary.
[28] Dependent variable: See glossary.
[29] Constants: See glossary.

11. Conclusions

When I first chose the topic for this research project I was quite worried I could not really achieve some ideas I had in mind. I knew from the beginning it would be hard to actually carry out an exploration like this since it was, and it still is an innovative subject. At the end, I actually managed to do so and completed my goals.

During the summer I was lucky enough to go to the center CRAG where I was introduced to this world. That was the start of my project and I personally do not think I could have ever gotten so far without my several visits to this center, in fact, that was the basis that put together all my work. On the other hand in order to be objective about this topic, I got in touch with the *Departament d'Agricultura de la Generalitat de Catalunya,* there I was again so lucky to get attended to. This association also provided me a lot of information and data that I afterwards used to complete my work.

As far as my practical framework I found so hard to prove and demonstrate all my work in a single experiment. My first idea for this was to actually create a GMO, by myself. However, since I did not have all the equipment needed at my disposal I found myself obligated to look for an alternative. After doing a lot of research, I decided to carry out an experiment that could actually be done at home and that represented a little part of my project. For this, I managed to extract the DNA out of an organism. It is true I enjoyed doing my practical framework more than the theoretical one but without this last one I could have not conducted the rest of my work. If I have had more time, I would have liked to continue researching about other laboratory techniques and grow a GMO.

I have to confess the fact I decided to do this research project in a different language had me quite concerned. However, I finally took courage and went ahead with it, and I am extremely satisfied for this. This research project has helped me become aware of something I did not know much about, it has helped me comprehend both of the perspectives towards the polemic of the subject, and it has finally taught me how to carry out an exploration like this kind. I am extremely grateful for completing these goals and so thankful for everyone that has dedicated even a minute of their lives to helping with this project and I can finally say that I feel so proud I have done something I am so delighted with.

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13. Appendix 1: CRAG CRISPR9

[30]CRISPR (Clustered Regularly Interspaced Short PalindromicRepeats). The prokarytic adaptive immune response
A continuación vamos a analizar el funcionamiento de CRISPR: La respuesta de la inmunidad adaptativa de bacterias:

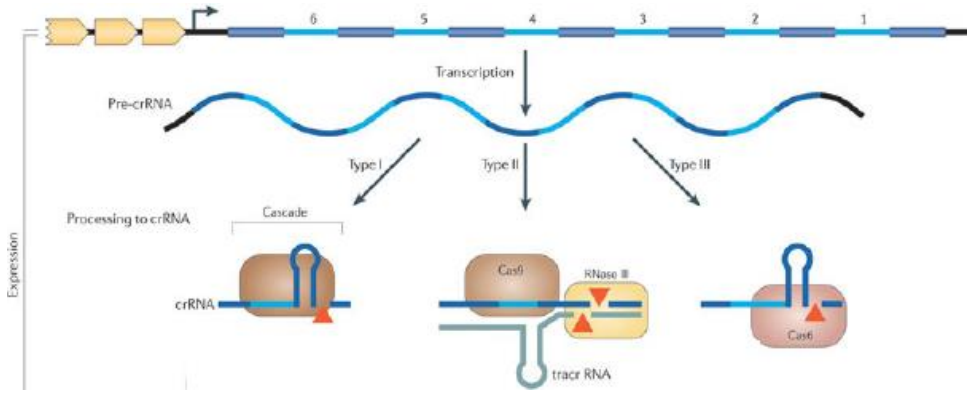
Paso 1: Adquisición de nuevos espaciadores
Cuando se captan nuevos espaciadores procedentes del DNA extraño, este se introduce dentro de la bacteria (después de ser degradado por Cas1 y cas2). Lo importante es cuál de las moléculas es la que forma parte como nuevo espaciador. Estas nuevas secuencias incorporadas poseen además el motivo PAM (adyacente al espaciador). De alguna forma esto permite discriminar entre lo propio y lo extraño. Preferiblemente se integran moléculas de ácido nucleico.
Estas proteínas Cas1 y cas2 cuando lo degradan integran ese espaciador nuevo que va a ocupar el primer lugar dentro de la secuencia CRISPR (Ver en la última imagen de la página anterior el Número 0 en rojo). La moraleja es que este locus nos da una idea de la historia. Las secuencia incorporadas más tarde (las más nuevas) estarán delante y las más viejas detrás.

Es importante la orientación de ese espaciador dentro de los locus CRISPR. Sólo en la correcta orientación son capaces de dirigir el DNA extraño mediante el reconocimiento del PAM en el extremo 5´. La secuencia PAM, para un mismo microorganismo siempre tiene que estar orientado en la misma dirección. Para cada organismo puede estar de una forma u otra, pero todos en la misma dirección. Si se inserta en la orientación adecuada hará función biológica, si no, a la hora de interactuar con el DNA extraño no lo reconocerá.

Una vez integrado, y generada la memoria inmune, a partir de la secuencia líder se transcribe un RNA muy largo consistente en espaciador-repetición…etc. Esto, para ejercer su función se tiene que procesar. Antes de este procesamiento se llama Pre-crRNA (Se dice: "Pre-crisprRNA"), que al final se convertirá en el crRNA ("crisprRNA"). De momento el Pre-crRNA es una molécula inmadura que ha de procesarse.

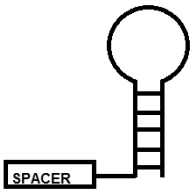
[30] This information has been extracted from Miguel Ezquerros' thesis for his doctorate.

Ahora conviene saber que existen diferentes sistemas de CRISPR, cada uno con sus proteínas propias. No hemos de saber todos ellos, simplemente saber que hay más de uno. En función de esos tipos se va a procesar ese Pre-crRNA con unas proteínas o con otras, esa es la diferencia. Ej (solo para aclarar): en el caso del tipo I, es la proteína Cascade la que reconoce las repeticiones que son capaces de formar estructuras secundarias (horquilla), las reconoce y corta al final de la horquilla. Como corta al final nos va a hacer que tenga diferentes subunidades cada una de ellas con un espaciador. Nosotros principalmente vamos a hablar del de **TIPO II**, que es el más utilizado:



Paso 2: Expresión de crRNA. Sistema tipo II

En las de tipo II interviene la proteína **Cas9** (nucleasa), pero además de ella, hace falta la intervención de un segundo tipo de RNA, que es el **tracrRNA** (trans-activating crRNA). Este es complementario en parte a una de las repeticiones que tiene la secuencia de crRNA y tiene además una horquilla, lo cual hace que cas9 corte donde tiene que cortar, es decir, donde se aparean los dos RNA, dejando (al igual que antes) un espaciador flanqueado por la secuencia de repetición.



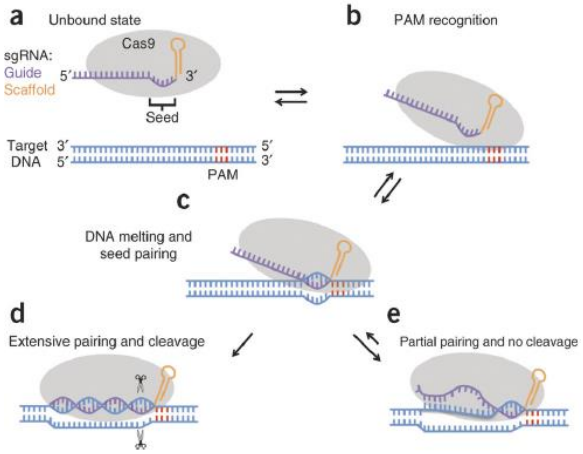
En conclusión, cuando estos crRNA se hibridan con cas 9 y el tracrRNA, cas 9 rompe los RNA y deja el crRNA maduro. Finalmente quedan la secuencia de repetición y el espaciador. Nota: Siempre la repetición es la que va a formar una horquilla (en el caso del tipo I y tipo III también) y tendremos siempre un espaciador seguido de una horquilla (ver imagen). En el caso del tipo II no la forma porque está apareado con el tracrRNA que evita que la forme.

El tipo III es igual que el Tipo I, pero usa la proteína Cas 6, que corta detrás de la horquilla. Por tanto el Tipo I y III cortan al final de la horquilla y el tipo II corta donde tiene que cortar, donde le dice el tracrRNA, que actúa de guía.
Bien, ahora tenemos los **crRNA maduros**, es decir, hemos pasado de un péptido muy largo a tener cachitos que tiene la configuración de espaciador–horquilla (Ver imagen anterior de nuevo). Tantos cachitos como espaciadores tengamos.

Paso 3: Targeting Invading DNA/RNA (TYPE II CRISPR)

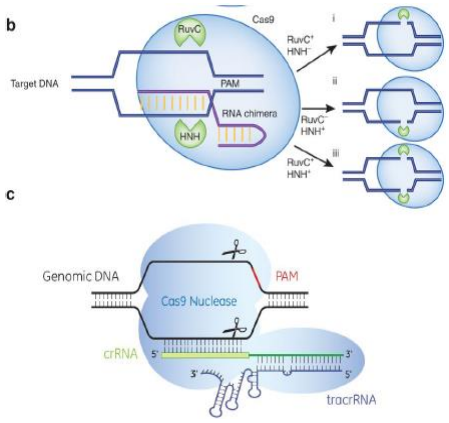
El siguiente paso es que esos espaciadores (complementarios a un DNA que pueden ser invasor) ataquen al DNA/RNA invasor.

¿Cómo lo ataca? Hay varios sistemas de control para que ataque a lo extraño y no al propio (hay que acordarse de la secuencia PAM). Esas dos unidades (Espaciador + horquilla) las reconoce cas9, puesto que reconoce la horquilla (la horquilla es común a todos los espaciadores). La propia cas9 reconoce el motivo PAM, así que preferentemente va a ir al DNA invasor. Si se equivoca y va al DNA propio, la secuencia del espaciador no será complementaria al mismo por lo que no va a poder atacarlo.



La proteína cas9 cargada con el crRNA reconoce el motivo PAM, se hibrida con él y como la secuencia del espaciador puede ser complementaria a ese DNA con el que se está apareando la proteína Cas9, tendremos dos opciones:

- Si el DNA es extraño habrá apareamiento completo, si lo hay lo rompe ya que es una endonucleasa.
- Si por alguna razón Cas9 reconoce DNA propio, raro va a ser que el espaciador reconozca el DNA propio, es decir, va a ser muy difícil que tanto el PAM como el espaciador sean reconocidos como propios. Así que no va a haber apareamiento de bases y por lo tanto no va a actuar.



¿Cómo se degrada ese ácido nucleico? Se degrada porque la proteína Cas9 tiene dos actividades nucleasas, tiene dos dominios nucleasas: **RuvC** y **HNH**. Diferencia entre los dos:

- La **RuvC nucleasa** rompe la cadena de ácido nucleico no diana, es decir frente a la que no se está alineando el crRNA (En violeta) (El DNA alineado está en azul oscuro). Entonces, la cadena no diana la rompe el dominio RuvC.
- La cadena diana, es decir, a la que se está alineando el crRNA la rompe el dominio **HNH**.

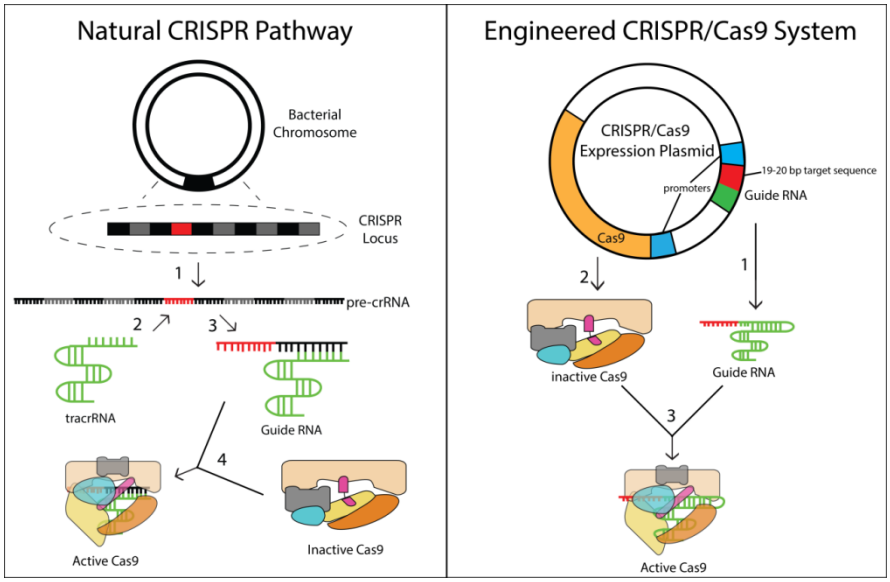
  Esto se ha hecho haciendo ingeniería de proteínas. Se ha visto que mutando uno de los dos dominios, una de las cadenas quedaba intacta y la otra se rompía, así se ha visto.

Nota: En los demás tipos de CRISPR (Tipo I-A, Tipo I-E, Tipo II, Tipo III-A y Tipo III-B) no hace falta un tracrRNA, este solo se utiliza en los de tipo II (como ya comentamos).

Aplicaciones de CRISPR

Gene Knock-out

Tenemos una bacteria con el sistema CRISPR-CAS y queremos usarlo para hacer un knock-out de esa propia bacteria. Tendríamos que **meter un nuevo espaciador (y una nueva repetición) introduciendo en él la secuencia del gen que queremos inactivar en la bacteria**. Habrá que buscar una región PAM y todo para que funcione. Esto se ha hecho y es fácil pero con microorganismos que se dejan manipular bien. Para bichos que se manipulan mal lo que se está haciendo es usar un plásmido que solo tiene que expresar Cas9 (en naranja) y en la otra zona del plásmido el promotor (en azul claro), para que se exprese bien Cas9, con la secuencia guía de RNA, es decir, el crRNA (en rojo-verde). Así que en el plásmido tiene todo para que funcione el sistema Crispr, todo lo demás lo pone la bacteria (cas1, cas2, espaciador…)

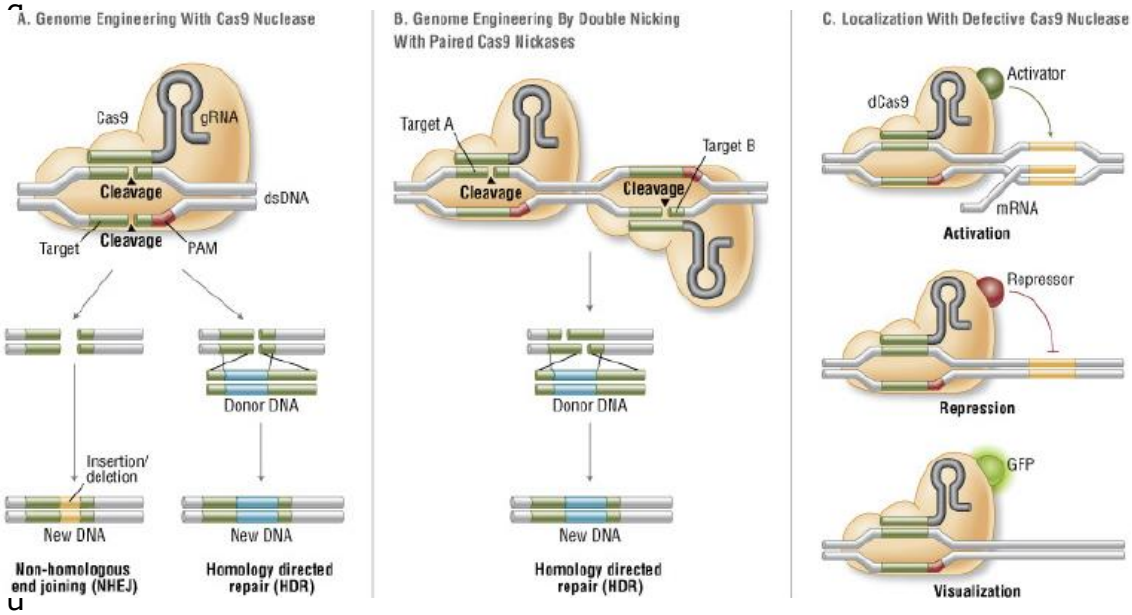


Genome Editing. Edición del genoma en Eucariotas

A. Si queremos inactivar un gen (hacer un KO) basta con meter un espaciador o bien clonarlo en un plásmido (ver punto anterior) para que CRISPR ataque. Se genera knock-out porque al repararse la mella creada por Cas9 no se repara de manera igual a la original, sino que hay una delección o inserción de un nucleótido que hace que se rompa el marco de lectura y el gen no se constituye de manera funcional como lo estaba inicialmente; o bien porque una vez que cas9 rompe, se introduce una inserción o una delección, es decir, un fragmento de DNA que tenga la delección con extremos homólogos a los que dejaba Cas9 y por recombinación homóloga esto va a dar lugar a un nuevo cromosoma. (Esto es útil en terapia génica).

B. Otra manera es introducir una nucleasa (Una proteína Cas9) que solo ataque a una de las cadenas, entonces se forma una mella en una cadena solo (Eso se hace mutando unos de los dominios nucleasas). Usamos una proteína Cas9 con un crRNA en una parte del cromosoma que haga una mella en esa parte del cromosoma. Seguidamente usamos otro crRNA para que introduzca otra mella en otra parte del cromosoma. Al hacer dos mellas se puede separar esa doble cadena de DNA y podemos repararla a nuestro antojo por recombinación homóloga.

C. Para hacer estudios de función se hacen proteínas cas9 inactivas, es decir, que no tienen actividad nucleasa. Pero aunque no tengan dicha actividad nos van a permitir posicionar esta proteína Cas9 donde queramos. ¿Cómo? Porque el CRISPR-RNA nos la lleva a donde queramos. Entonces podemos meter en la proteína Cas9 un activador genético (ya que hay un factor de transcripción que no se activa). Por tanto lo que va a pasar es q



ucirá la activación del gen que tiene al lado. Esto es útil en enfermedades humanas en las que no se produce la proteína. Se puede hacer lo mismo con un represor o para ver dónde están localizados los genes con un reportero.

Interference with transcription (CRISPRi)

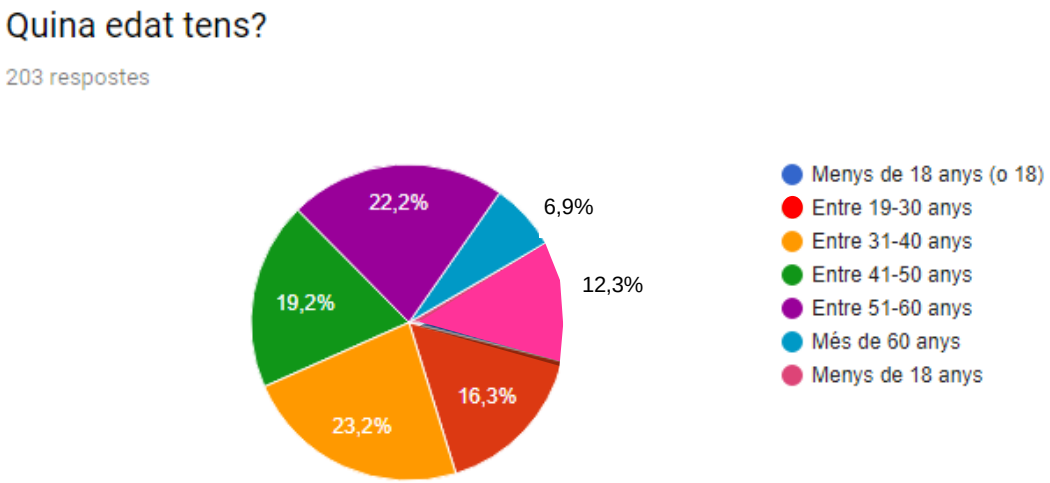
En relación al último caso (Cas9 sin actividad nucleasa) recientemente se ha desarrollado la interferencia por CRISPR (CRISPRi), que no es útil para hacer un KO, pero sí para evitar la transcripción de transgenes. ¿Cómo lo hacemos? Metemos Cas9 acomplejado a crRNA (Cas9 no tiene actividad nucleasa por lo que se queda ahí), llega la RNA polimerasa para transcribir el gen, pero como eso está ocupando ese espacio no puede transcribir el gen que nos interesa silenciar.

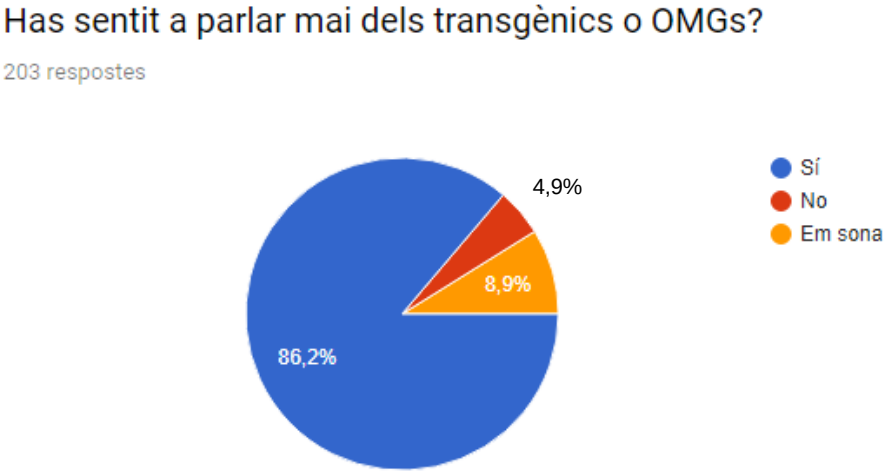
14. Appendix 2: Random survey

The main objectives of doing this survey were to find out about the grade of information people have about the genetically modified organisms. With the aim of learning, I passed around, via online, a survey consisted of 6 questions. People who responded to my survey were asked many questions, from the classification of their age to specific questions about the GMOs.

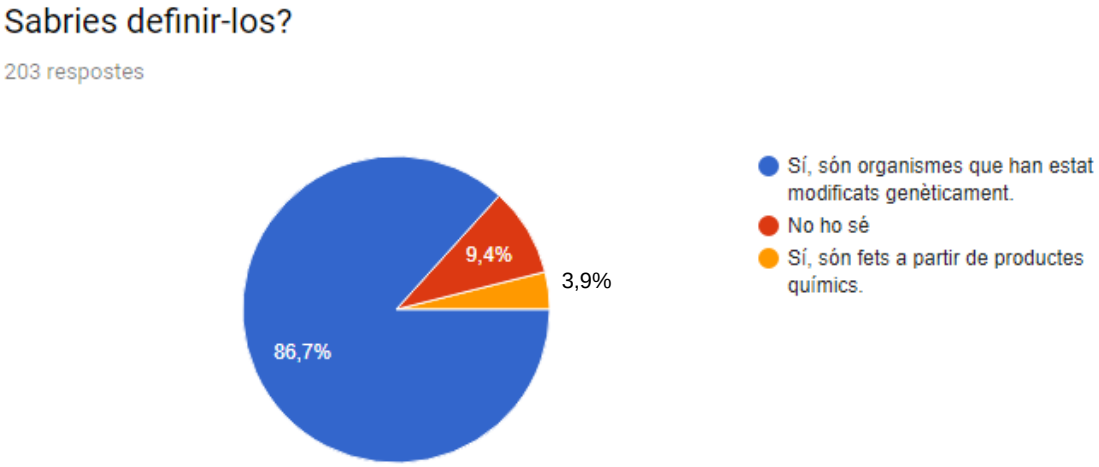
Taking all the data into account, I received 203 answers. Out of all of these answers, I managed to obtain quite representative answers since the ratios of age overall are pretty regular. I got answers from people surpassing the sixties which was a success since I did not expect to reach that far because of the technology channel.

The following graphs show the answers with a percentage respectively as well as the questions going with them.

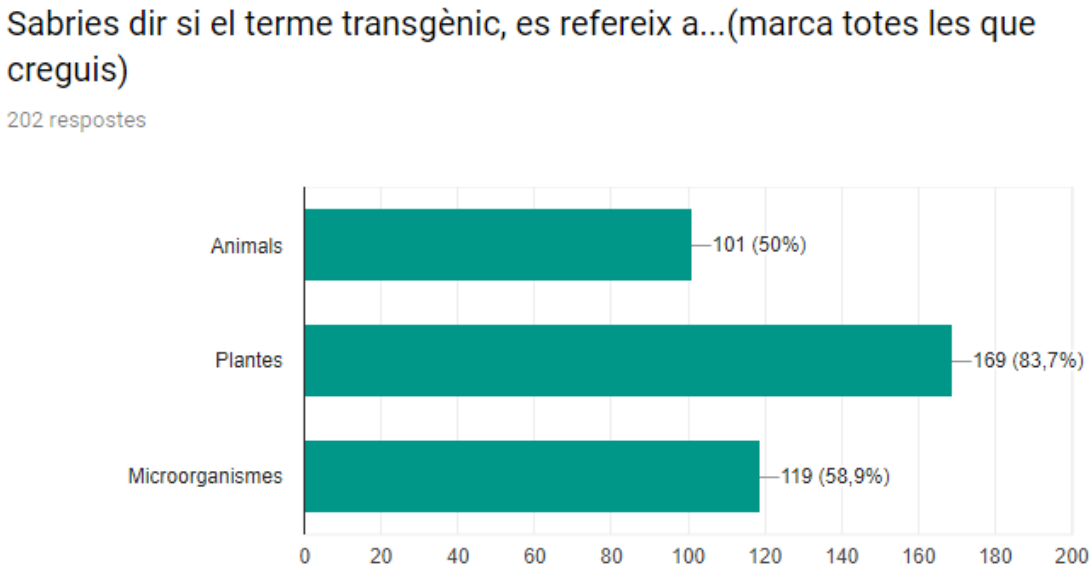




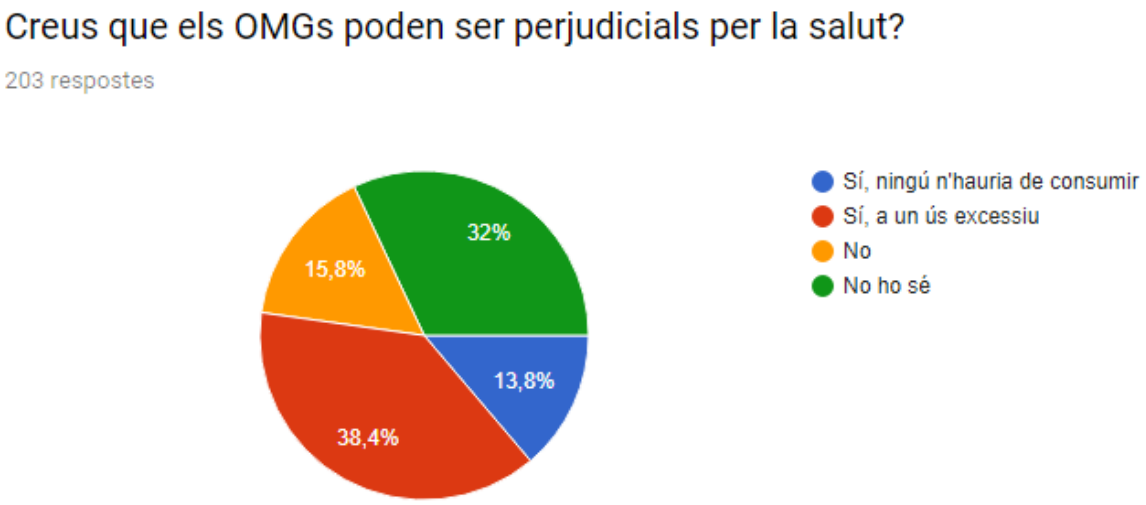
The results to this question show how above the majority of the people (86,2%) have actually heard about GMOs.



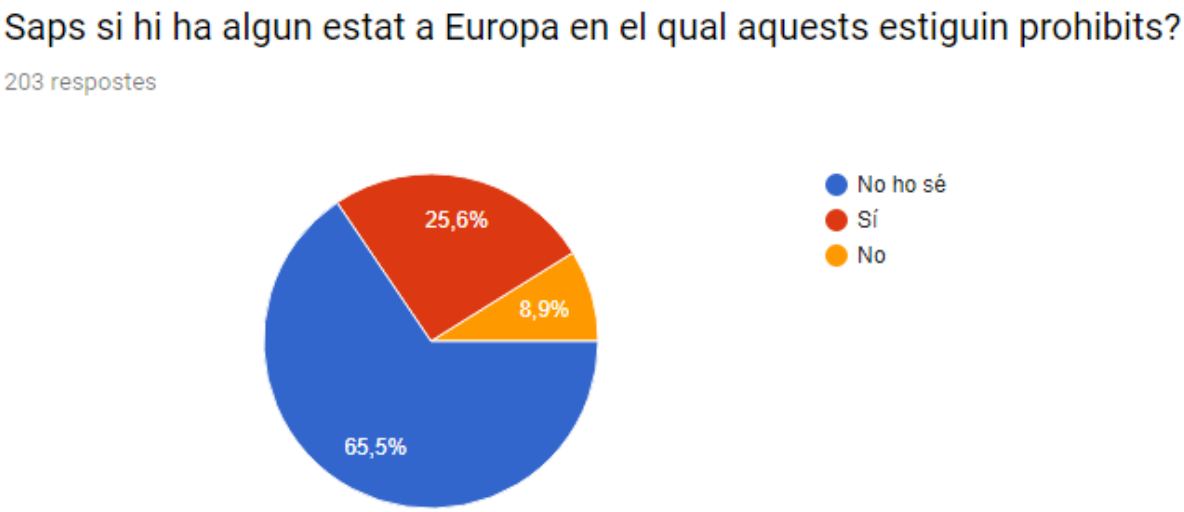
The results to this question actually surprised me since I did not expect that much percentage of people (86,7%) knowing what GMOs were and how to define them.



This is an interesting question since most people think GMOs are just plants, in the graph shown above 169 people thought genetically modified organisms were plants which is true, but the real truth is that they can be all; animals, plants, and microorganisms. And this graph shows not everyone has this idea clear in their minds, otherwise the graph would be evenly-matched.



The opposition to GMOs remains high and most people (52,2%) think they are harmful for human health. On the other hand there is also a big range of people who remain neutral and do not have an opinion in the subject (32%).



In this graph, it is shows how 65% of the people who responded to the survey did not know about the legislation in Europe and answered they did not know whether GMOs were prohibited in some area of the European Union. This shows there is a lack of information towards people about this topic.

15. Appendix 3: Tomato protocol

1. Prepare the Microtome seeds for cultivation in MSO[31] (Murashige and Skoog medium) culture media at 50%.
Duration: 10 days.
Temperature: 25ºC.
Light: Examination lamps.

2. Cut the cotyledons and place them in KCMS[32] (Keratinocyte culture medium).
Duration: 24 hours.
Temperature: 25ºC.
Light: Darkness.

3. Transform and place the cotyledons in KCMS culture media.
Duration: 48 hours.
Temperature: 25ºC.
Light: Darkness.

4. Place the cotyledons in 2Z[33] culture media.
Duration: 15 days.
Temperature: 25ºC.
Light: Examination lamps.

5. Sub cultivate the cotyledons in 2Z culture media.
Duration: 15 days.
Temperature: 25ºC.
Light: Examination lamps.
6. Sub cultivate the cotyledons in 2Z culture media.
Duration: 15 days.
Temperature: 25ºC.
Light: Examination lamps.

[31] The media is explained on page 55.
[32] The media is explained on page 55.
[33] The media is explained on page 55.

7. Rooting media.

Duration: X days.

Temperature: 25ºC.

Light: Examination lamps.

8. Acclimatization in the greenhouse.

9. Greenhouse → seeds (2-3 months).

TYPES OF CULTURE MEDIA:

MSO culture media at 50%

This culture contains:

- MS salts at 50% =2'15 g/l.
- Sucrose 30 g/l.
- B5 Vitamins including Inositol (100 mg/l), Nicotinic Acid (1 mg/l), Pyridoxine (1 mg/l, Thiamine (10 mg/l).
- Agar Difco 8 g/l.
- pH = 5'8 KOH.

KCMS culture media

This culture contains:

- MS salts and vitamins.
- Sucrose 20 g/l.
- KH_2PO_4 200 mg/l.
- Thiamine 0'9 mg/l.
- 2'4 D 2 mg/l.
- Kinetin 1 mg/l.
- Acetosyringone 200 micro molar.
- pH = 5'8 KOH.
- Agar Difco 8 g/l.

2Z culture media

This culture contains:

- MS salts.
- Sucrose 30 g/l.

- Nitsch Vitamins including biotin (0'05 mg/l), folic acid (0'5 mg/l), glycine (2 mg/l), Inositol (100 mg/l), Pyridoxine (0'5 mg/l), Thiamine (0'5 mg/l) and Nicotinic Acid (5 mg/l).
- Zeatin (plant hormone) 2 mg/l.
- Timentin 250 mg/l.
- Kanamycin 100 mg/l.
- pH = 5'8 KOK.
- Agar Difco 8 g/l.

In the second culture of the cotyledons in the 2Z culture media it is important to reduce Timentin's level to 150 mg/l. In case of the presence of Agro contamination, the concentration must be reverted to the initial one, that is to say 20 mg/l.

Rooting media

This media contains:

- MS salts at 50%.
- Sucrose 10 g/l.
- Nitsch Vitamins.
- Timentin 75 mg/l.
- Kanamycin 100 mg/l.
- pH = 5'8 KOH.
- Agar Difco 8 g/l.

For the transformation, it is important to cut the cotyledons in a half and get rid of the borders or ends and place them into plaques with KCMS culture media (with the underside in contact with the culture media) during 24 hours at 25ºC and darkness. Then, the cotyledons are soaked within the ***Agro*** solution, which contains *Agro* with KCMS liquid culture media, during 30 minutes and smooth stirring (around the 50 rmp[34]). Afterwards, the *Agro* solution is eliminated and the cotyledons are dried with sterile filter paper and paced in KCMS culture media. Here, the underside of the cotyledons needs to be in contact with the media during 48 hours at 25º and darkness.

[34] Rpm: See glossary.

For the sterilization of the seeds, it is necessary to place them on a Petri dish (Sterilin) with sterile H_20 (sterile water) during 30 minutes in a laminar flow cabin. Then, the water is eliminated and the following solution, Captan (3g/l) is added for 5 minutes. It is important to: get rid of the fungal solution, wash repeatedly the seeds with the presence of sterile H_20, and add the sterilization solution and leave it like this for 30 more minutes. After this, it is important to carry out three consecutive washes with sterile H_20 in minute 5, 10, and 15.

Seeds need to be planted into the pitchers with the germination media and place them in the culture camera "in vitro" of 24ºC and covered in aluminum foil and darkness.

After 2 days, the aluminum foil is taken out and the plaques are left exposed to light. In CRAG the solution used for sterilization contains 40% of sodium hypochlorite (NaClO).

16. Appendix 4: Punt de vista del Departament d'Agricultura de la Generalitat de Catalunya

Unió de Pagesos es va mostrar l'any 1997 en el seu VII Congres celebrat a Figueres, els seus dubtes davant la nova tecnologia dels transgènics, perquè la pagesia no disposava d'informació suficient al respecte per poder avaluar el seu impacte sobre l'activitat agrària i els seus efectes mediambientals, socials i econòmics.

Posteriorment, l'any 2004 en el seu IX Congrés celebrat a Tortosa i Amposta Unió de Pagesos apostava per una Catalunya lliure de transgènics perquè:

- És una tècnica que no es domina en la seva totalitat.
- Es desconeix el seu impacte en l'ecosistema i els sòls.
- Considerem que l'agricultura convencional i l'ecològica no ha de suportar els costos o lucre cessant per evitar la contaminació amb OGM.
- No es coneix com evitar la contaminació dels OGM amb l'agricultura convencional i ecològica.
- Impossibilitat de les finances catalanes per assumir el cost econòmic que suposaria mantenir les tres agricultures (convencional, transgènica i ecològica).
- Impossibilitat d'establir la responsabilitat d'origen de la possible contaminació transgènica.

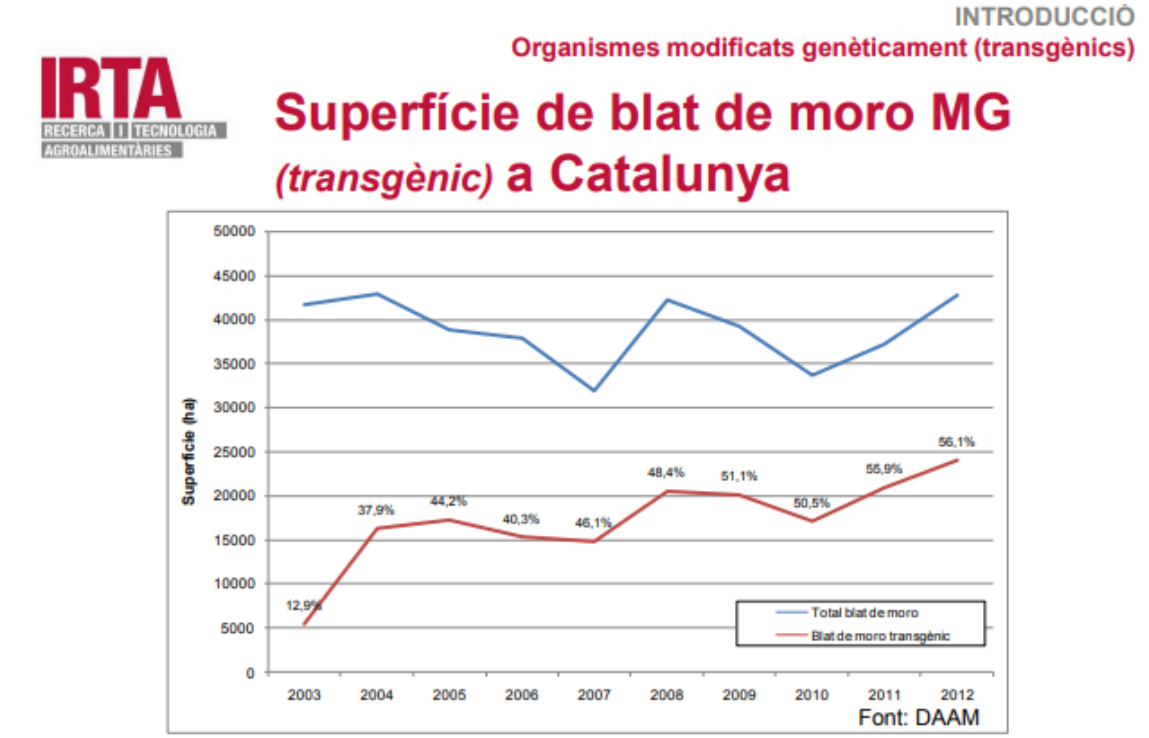
Per això, apostàvem perquè es fes respectar les normes del dret civil català i la qualitat agroalimentària, mentre no fos possible declarar dins d'Europa una zona lliure de transgènics.

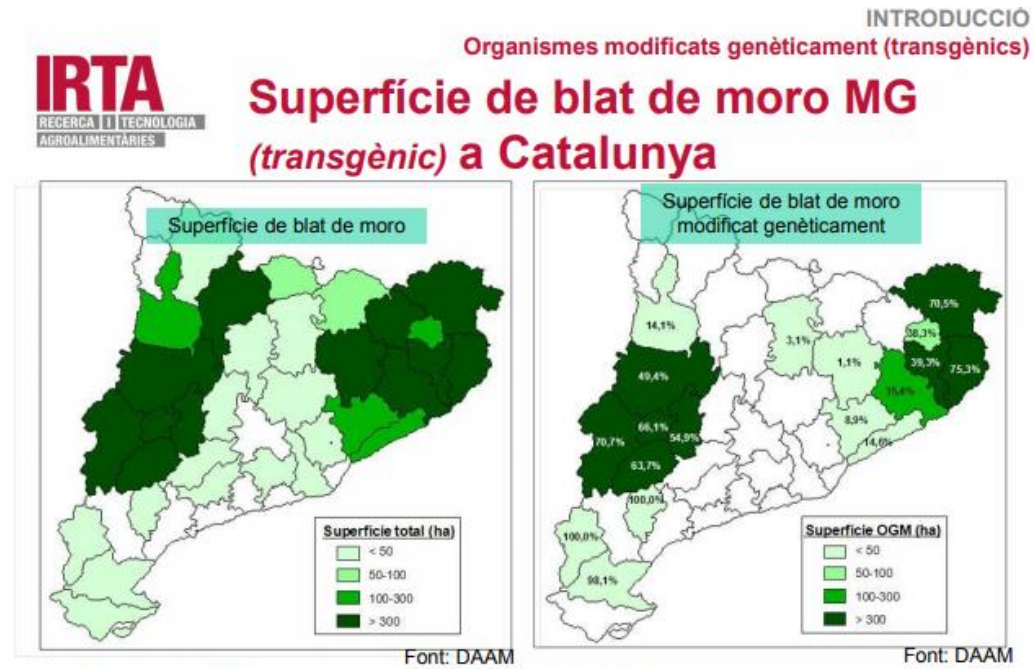
Posteriorment, l'any 2008 en la resolució Sobre el foment de l'agricultura i ramaderia ecològiques del X Congrés celebrat a les Borges Blanques continuàvem apostant perquè a Catalunya no hi hagin productes transgènics però remarcàvem el nostre respecte per aquells companys i companyes pagesos i pageses que en fan, deixant clar que la llibertat d'elecció és un dret.

En la 10a Assemblea celebrada a Olot l'any 2010 acordàvem que mentre la normativa permeti el conreu d'OGM la Unió de Pagesos cal que s'impliqui en un veïnatge pacífic i treballi per un marc jurídic clar que permeti la convivència de cultius ecològics, convencionals i transgènics, tot garantint la lliure elecció de

cada pagès i un etiquetatge clar que permeti l'elecció dels consumidors fins al final de la cadena de transformació. També, continuàvem apostant per un producte alimentari de qualitat diferenciada com a lliure de transgènics.

En la 11ena Assemblea celebrada a Martorell el 2014 ens vam reafirmar en aquest posicionament: lliure elecció de la pagesia, marc jurídic clar que permeti la convivència, un etiquetatge clar i una marca de qualitat diferenciada pels productes lliures de transgènics. A continuació hi ha alguns valors remarcables.





17. Glossary

Aleatory: Changing something that is being created.

Amino acids: They are the building blocks of proteins.

Crown gall: Crown gall is identified by overgrowths appearing as galls on roots.

Constant: In an experiment, the constants are those values that remain the same in spite of the variables.

Cotyledon: It is a significant part of the embryo within the seed of a plant.

Dependent variable: Those values that change as a response to the independent variable.

Dicotyledon: Also known as dicots, these are flowering plants whose seeds contain two embryonic leaves or cotyledons.

Enzymes: A substance in living organisms made up of protein molecules that act as a catalyst. Enzymes accelerate reactions and they are specific (each reaction needs its own enzyme). It is easy to distinguish enzymes from other words when reading since its nomenclature says they are always going to be finished by the suffix –ASE or -OSE.

Fungus: Is any member of the group of eukaryotic organisms that includes microorganisms such as yeasts.

Genetic engineering: Direct manipulation of an organism's genes using biotechnology.

Greenhouse: Structure with walls and roof, in which plants requiring regulated climatic conditions are grown.

Hybrids: It is the result of combining the qualities of two organisms of different breeds, varieties, and species.

Independent variable: Manipulated values in a controlled way to provoke changes into the dependent variable.

"In vitro": Studies are performed with microorganisms, cells, or biological molecules outside their normal biological context.

Kingdom: The second highest taxonomic rank, just below domain.

Monocotyledon: Flowering plants whose seeds typically contain only one embryonic leaf, or cotyledon.

Phenotype: Expression of the genotype modulated by the interaction with the media.

Pseudomonas spp: Type of bacteria.

Recombinant DNA (rDNA): These are DNA molecules formed by laboratory methods of genetic recombination to bring together genetic material.

Rpm: Revolutions per minute.

Tumor: An abnormal mass of tissue that results when cells divide more than they should or do not die when they should.

Tungsten: Also known as Wolfram is a chemical element with symbol *W* and atomic number *74*.

Vector: An organism that does not cause disease itself but it spreads infection by conveying pathogens from one host to another.