Genetic Engineering in Agriculture

Genetically Modified Seeds and Crops

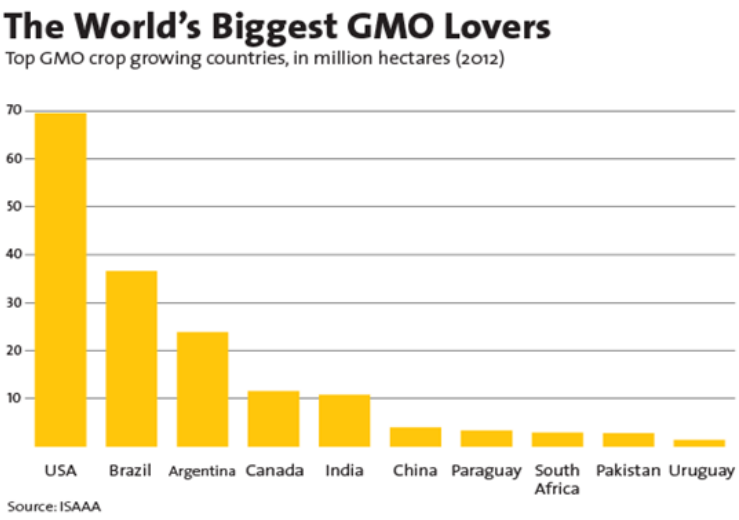
Preface

The Earth's population is growing drastically, with that the demand for food is increasing, cities are getting bigger, there is a decreasing amount of land available for agriculture. Therefore it is getting more and more important to think about the most efficient ways of producing large amounts of food. Are genetically modified crops the solution ? What if there are negative side effects (e.g. health related ones)? This is why we are interested in learning more about genetic engineering in agriculture and also why we chose this topic. In the past few years science has already gained and developed a lot of new knowledge and innovative techniques and there are still many researches going on in this field around the globe. We wanted to have a closer look at how the technology works, what the public and the law says about it in Switzerland and what advantages and disadvantages there are. Furthermore we were curious about what creative or witty experiments had been done and what hopes there are in genetic engineering in agriculture.

Introduction

More and more people are looking for the label "GMO free" while grocery shopping. In Switzerland there have been multiple votes about whether or not genetically modified plants should be allowed in Swiss supermarkets. Here it is important do differentiate between allowing GMO in foods which people eat and allowing our farm animals to eat GMO. In 2017 the Council of States in Switzerland chose to extend the law which forbids sowing and growing genetically modified plants in Switzerland for another four years. In comparison GMO has already been planted for years in Australia. On February 13th 2018 the Australian company *Nuseed* received the world's very first cultivation approval for omega-3 fatty acid enriched rapeseed. This is a big step for agriculture and for food industries, because there are already 83% of the world's population who eat too little omega-3 fatty acids according to the *World Health Organization*. The reason for this is the overfishing in oceans.
In 2016 there were 16 countries who cultivated genetically modified corn on a total land size of 60 million hectare. This number corresponds to 25% of the global corn acreage. Biotechnology is not a brand new concept; it developed over time. More than 10'000 years ago people started to domesticate crops. After that people began to do selective cross breeding. In order to have the best crops possible, they selected the plants with the prefered traits and either bred the best ones or crossed two closely related plants, to combine the traits. In 1940 the concept of mutagenesis was found. The idea behind this is to change a plant's DNA with the help of chemicals or radiation which results in a mutation. The more modern technology of genetic engineering by gene transferring was developed in the late 20th century.
There are traits that you cannot produce by using conventional plant breeding, i.e. if the trait which you want to have in one species is in another species. The solution of this problem cannot be solved through conventional methods. This can be done with genetic engineering! The main reason why farmers use genetically modified seeds is

because of the profitable traits the crops will have. The farmers may use less or in some cases no insecticides at all, if they have an insect resistant crop, or they get better weed control through herbicide-resistant crops. There are a lot more examples for desirable traits : virus-resistance, bacterium-resistance, fungus-resistance, severe weather condition resistance, faster growth, etc… As a result, the production of the farmers is less expensive and more efficient. The benefits of GM-crops and seeds cannot be gained from traditional, conventional methods, such as cross breeding. For example, in 2005, researchers at Syngenta innovated Golden rice 2, which contains much more carotenoids and beta-carotene than normal rice.
In this diagram you can see that the world's biggest user of GMO is the USA, South America and Asia. The USA is the largest grower of genetically modified crops in the world; they sell 90% of the world's genetically modified seeds. Brazil is the second largest GMO producer. A total of 93% of their soybeans are genetically modified. However in 2017 Brazil banned GMO imports from the US, probably due to trading reasons. In many countries in Africa and Europe it is against the law to grow genetically modified plants. In 2015 there were 38 countries who had banned GMO, 50% of which were European countries.



The Techniques

There are three different ways of modifying seeds and crops. The first option is to insert a gene from one species into another species. This is called transgenic modification. On the other hand if a organism with a certain characteristic is developed using genes of another organism of the same or a closely related species, the method is called cisgenic modification. The third way of modification is called subgenic modification. Instead of adding or replacing a gene in a plant, in the techniques of subgenic modification it is possible to remove or delete a gene
(gene knockout/ gene knockdown).

There are several basic steps in the process of making a genetically modified organism; identify/locate, isolate/clone and insert.
In order to modify, add or remove a gene it is mandatory to first identify and locate the desired gene. To identify the gene genetic screens can be made. DNA is randomly being mutated, until the sought-after gene is found. By comparing inheritance with already known locations of genes it is possible to map the exact position of a gene. In this process the phenotype of a species is examined to find out which gene is responsible for a certain characteristic -forward genetics. In contrast reversed genetics is done by observing a gene which already has been identified to witness how it evolves in a phenotype and under which circumstances it becomes active.

After identifying the gene, a cell has to be opened in order to take out the DNA. The DNA has to be separated from other substances of the cell. This can be done using centrifugation. Once the DNA is taken out, the specific gene has to be cut out with the help of restriction enzymes. Restriction enzymes are enzymes which can split up a DNA near or even exactly at the recognition sites. If the sequence of a gene is known the sequence can be reinforced by using polymerase chain reactions. However, today a gene can be artificially synthesized if the necessary data is available. The DNA fragments are then put into plasmid vectors and grown in bacteria, where they also are multiplied as the bacteria divides. The next step is to transfer the DNA into a cell. In agriculture there are four main methods. The easiest one is Agrobacterium. Cut plant tissues are soaked in a fluid with Agrobacterium inside. As the plant tissues are cut, the agrobacterium can attach to many of the hereby exposed plant cells. The Agrobacterium contains a protein that has nuclear localization signals, which helps it to direct the t-DNA to the nucleus of the cell. The plant tissue, which has been modified now, will now be cultivated on a selective medium to create shoot. The shoot is then put into another medium to form roots. These roots can then be grown like ordinary plants and the seeds from these plants are then planted on a selective in order to remove the wildtypes.

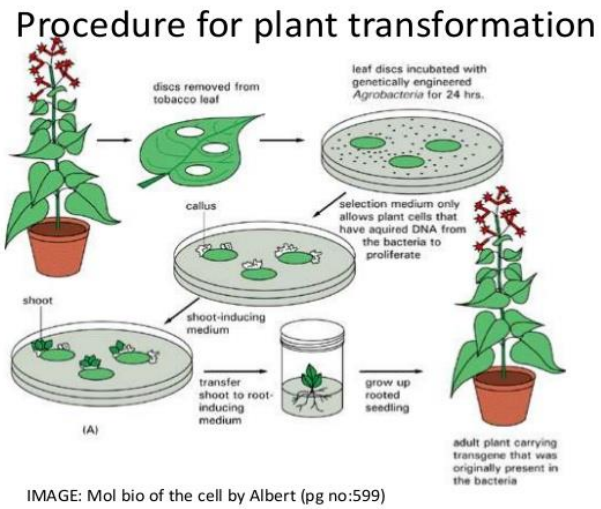
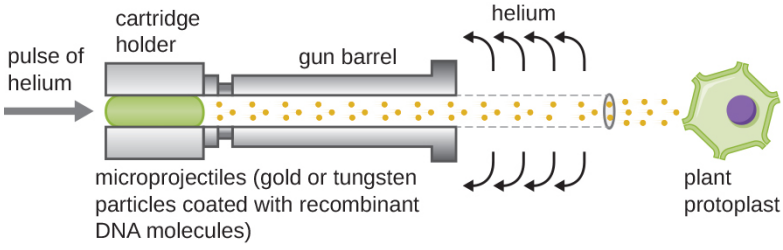


IMAGE: Mol bio of the cell by Albert (pg no:599)

The second method could also be called a transfection rather than a transformation, as the added genes do not necessarily reach the cell's nucleus. This method is called Viral transformation. In this process a chosen plant virus is modified by concentrating the prefered genetic materials into it. The virus will then infect the plant. Most of the time the genetic material occurs in the form of RNA. In this case the RNA will replicate itself in the cytoplasm of the targeted cell. A disadvantage of this method is, that the next generation won't contain the previously inserted genes.

Genes from one organism can also be inserted into another organism with Gene guns. Here particles of i.e. gold are coated with DNA. With the gene gun the genetic material is then shot into a plant cell. Some of the genetic materials will stay and transform themselves in the cell. This method is less efficient in transformation than Agrobacterium.

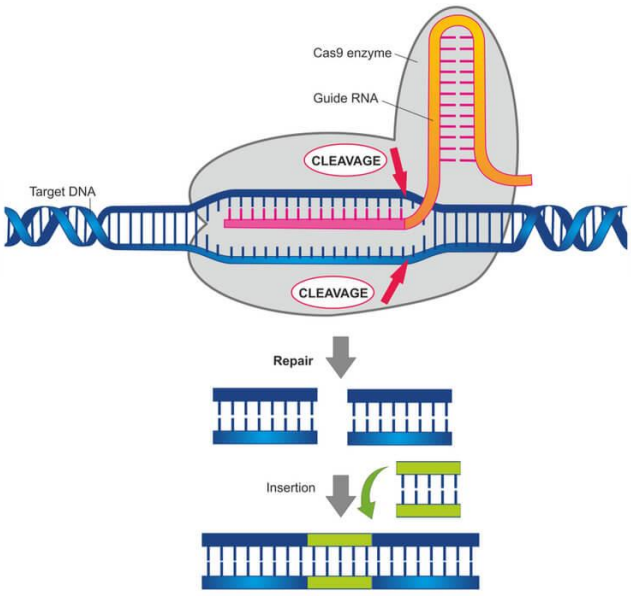
The fourth method is called Electroporation. This is not a method to transform genetic material, but a method to make it easier to insert genes into a cell. In Electroporation an electrical field is put on a cell, This makes the cell membrane more permeable and therefore more efficient to introduce new DNA.



Picture: Gene Gun

The last step is to select the modified organisms from the not modified ones. This is done by growing the cells in the presence of antibiotics or other chemicals which are able to mark the transformed organisms. After the successfully modified plants have been marked, there are multiple easy ways of separating the GMO's from the 'normal/original' plants.

CRISPR/Cas9 is another rather new gene editing method first published in 2012. With this method no other organism is needed than the one you want to modify as you directly cut the genes. This new method is based on a system found in bacteria. This system makes the bacteria produce short RNAs when they discover the presence of viruses. The RNA has a sequence which matches the sequence of the virus, the guide-RNA. Cas9 is an enzyme which has the ability to cut DNA. When the guide-RNA finds its matching sequence in the virus, the enzyme cuts the viral DNA precisely where the sequence was found. Researchers found a way to engineer this natural occuring system to use it to edit genes directly their cells. The Cas9, in the nucleus of a cell, will unzip the DNA at a predetermined DNA-sequence, so that it can connect with the RNA-sequence. When this is done the Cas9 will use two tiny so called molecular scissors to cut the DNA. The cell will try to repair the DNA and the enzyme cuts the DNA until a random mutation is formed. If you do not want a random mutation, but a precise edit, you can add another piece of DNA with your prefered sequence to replace the mutant DNA with this chosen DNA. This template can join the DNA, where it has been cut. With this new knowledge scientists can now do precise and multiple gene editings at once.



Picture: CRISPR/Cas9

Interviews

In the process of working on our term paper, we had the opportunity to talk to three different experts. In the following we will only write down some extracts of the discussions as all three interviews together would be three hours long.
Originally we had contacted the company *Bayer*. They then forwarded us to *scienceindustries*, where our first expert Dr. Jan Lucht is responsible for the area biotechnology. This interview was held in German, so what you will read below has been translated by us.
First he told us about the job situation in Switzerland: "Originally you contacted *Bayer*, but they passed on your mail to *scienceindustry* [...] there are not many experts in the field of biotechnology left in Switzerland. Most of them moved to another continent to work in the research department there. The reason is that the major markets of GM-products are in the Americas and China, due to this most of the research going on has also been shifted there. [...] *Bayer* and other biotech companies like *Syngenta* often

say they don't have time for interviews with the public, because all their experts are somewhere else. Therefore *Bayer* contacted me." We asked him, what he thinks why people especially in Europe have such a negative attitude towards GMO. "I think the people do not know enough about GMO [...] companies and people who actually have the knowledge about GMO, think that it is possible to change the people's opinion and take away their fear just by presenting the technology once. I think it is important to educate the people on this. [...] people might have seen a picture of some men or women on a field with a gasmask on and the letters GMO written on it, so every time they here something about GMO they automatically think about that picture. [...] in my opinion there should be options. I would like to see 'normal', organic and GM-food in the supermarkets [...] and everything should be labelled properly. Everyone should be able to decide by themselves what they want to eat [...] farmers should also have the right to choose what crops they want to grow." Then we wanted to know if there actually are negative consequences of GM-crops or if the things we heard about infertility etc. are only rumors. "There has never been any proof that women who eat GMO will be infertile [...] it is also false to think that genetically modified plants will spread out out of control. The only possible problem I know of is that if we use the same methods for a long period of time, eventually insects or viruses will get resistant to these GM-crops [...] we have found that nature is adaptable [...] we have to think longer term. This is the real difficulty."

For the second discussion we travelled to Stein to talk to Dr. Gabriel Scalliet, Team Leader Fungicide MOR at Syngenta. With him amongst other things we talked about what people know and what people think about natural products. He said: "I think that it is important that people understand a little bit about it [biotechnology]. [...] people actually think that what they see growing in the field is all natural, but it's not. It is all done by human and there is a lot of technology behind it [...] it's not random [...] yes, it is a natural process but the human was behind it all the time selecting for the best individuals [...] and nowadays it's faster, because we make use of the technology. We all know that too much chemicals is bad for the environment [...] with GM you might use less chemicals on the plants, which I think is a good thing. But it's difficult, people in general are against biotech." Then we talked about that there are no GM-foods in Swiss supermarkets and Mr. Scalliet answered: "Are you sure that there are no GM-crops? [...] you might have it in the cereals you eat and the meat, so pigs and chickens they get to eat soybean proteins from GM-soybeans. So yes, there are no direct GM-products, but it is hard to believe that there are no traces of GM [...] it is quite widely spread actually. I think what could have made the public's opinion more positive towards GM-crops could have been to start with other things than herbicide resistance. To have started with things that are more touching or closer to the heart of the people like that Golden Rice.

Last but not least we got the chance to talk to Dr. Michiel van Lookeren Campagne, President of Syngenta Biotechnology and Global Head of Seeds Research. He works and lives in North Carolina, therefore we talked to him over Skype. With him we mainly talked about the different technologies and when and how they are used. "We use Classical Breeding when we want to change the trait of crops and seeds [...] we use GM when there is no solution through breeding. The latest technology is genom editing, where we can speed up the breeding, because now instead of having to cross in the traits from a wild relative, we can just edit the existing gene. [...] these are new technologies, that are tools to create better seeds." Then we wanted to know from him with which technique they transfer the gene. He answered by saying: "The most

efficient way to transfer a gene is to use agrobacterium to transfer the T-DNA into the plants. And then what we do is, we select for plants that have taken up that DNA and then we grow a new plant out of it. And that is called plant transformation." Then we wanted to know more about the gene editing and he explained: "What we do with gene editing is, we use an enzyme that homes into a specific DNA sequence that we already predetermined and then it makes a cut. Then the repair mechanism of the plant tries to glue that back together [...] it cuts it so long until a mutation is created in that spot. Once there is a mutation it doesn't recognize the sequence anymore [...] then you have created an edit. Basically what gene editing does is, it causes a mutation, but not in a random way, but it does it very very specifically at a single location." Then we asked him how the T-DNA can know where to go. "You have to know at least 17-20 base pairs and then with the help of CRISPR/Cas9, the Cas9 is the a protein, the guide RNA brings the CRISPR to the precise location and then it makes a cut." We further asked what the most difficult part in genetic engineering is and his answer was: "The most difficult part is to regenerate the plants, because how do you make a plant out of one single cell, if the edit you make in one cell might be different from the edit you make in another cell." In the end we asked about current things Syngenta is working on. "We work on things like drought tolerance, nitrogen-use efficiency, disease resistance [...] basically it's all related to yield and productivity."

Discussion

Advantages:

The population is increasing dramatically. At the same time the demand for food is increasing too. The problem can be solved in two ways: Either we enlarge the farmland, so we have more space to grow crops on. But in this case we have to fell more trees and sacrifice natural reservoirs. Or we try to get better crop yield through genetically modified crops and seeds. The GM-Crops are more efficient, stronger and more profitable. It is easier to raise crops that are genetically modified, because they have a stronger ability to resist pests, insects, herbicide or severe weather conditions. This feature helps farmers to produce greater amounts of crops. Due to higher yield and lower costs of production, food prices would automatically go down. According to a statistic people in poorer countries spend over half of their income only on food, which means more poor people could afford food if the prices decreased. It has been proven that genetically modified crops do not need pesticides to become stronger against various types of insects or pests that may destroy them. The pest resistant trait leads to no use of pesticides. As we have mentioned, through genetically modified crops you have better yields, so you do not have to fell more forest. As deforestation is minimized, the deforestation rate decreases. This would again decrease the carbon dioxide in the atmosphere, which would slow down the global warming.

According to an Oklahoma State University report, the GM-Crops help to reduce greenhouse gas emission, soil erosion and environmental pollution. Therefore the general cleanliness, health and beauty of the environment are improved, which indirectly improves the well-being of the people.

Disadvantages:
Despite the advantages of genetically modified food we also have some disadvantages. According to research by the Brown University, current genetically modified food may result in allergic reactions in people. Through adding or mixing of proteins that were not indigenous to the plant, it might cause some new allergic reactions in the human body. The genetically modified foods combine proteins that humans were not used to consume it. It may increase the chances of an allergic reaction because: Since 1999, the rate of food allergies in children has increased from 3.4% to 5.1%. Another risk for the people is that, according to the Iowa State University, these genetically modified foods have an effect on the antibiotic efficacy. As crops are modified to resist to germs and pests, it may reduce the effectiveness of an antibiotic or other therapy. One of the biggest disadvantage that is being discussed is that the genetically modified food may also create new diseases. The justification is that GM-foods are modified using viruses and bacteria. There may be health risks on the consumption of certain GM-foods. The genetically modified crops and seeds also have disadvantages on the environment. It is known that the biodiversity is disrupted. Some organism in the ecosystem could be harmed, which leads to a lower level of biodiversity. The patents of genetically modified crops are controlled by six companies. Because of this, it is very difficult for independent researchers to have an influence in this field. About half of the GM-food producer do not allow any independent research on the crops and prevent researchers from finding out the long-term benefits or consequences that may exist from the GM-food. Another disadvantage is that genetically modified crops create unnatural, new combinations of DNA and these combinations could cause allergy activation, even if researches show that certain combinations are not harmful to humans.

Conclusion: Genetically modified foods are, because of their traits, very efficient in production, so we can have more yield without expanding the farmland. This can avoid the malnutrition and the undernourishment. Many hunger problems would be solved. The reliance to chemical pesticides and herbicides are reduced. At the same time we are protecting the environment. Because we do not have long-term experiences, we have to be cautious to avoid bad consequences to our health and surrounding.

Summary

Biotechnology has developed very much especially in the past few years. We have moved on from classical cross breeding, to genetic engineering with agrobacterium and now, since 2012 the efficient technology of CRISPR/Cas9. CRISPR/Cas9 made it possible to edit genes directly in the target cells and to do so with many genes at once, which accelerates the whole process and makes it more efficient. There are some disadvantages like allergies and the fact that farmers have to buy new seeds every year. But these problems might be solved in the future as researchers are working on them. In our opinion, however, the advantages genetic engineering brings overweigh the disadvantages by far. Even though GM-plants are not yet allowed in Switzerland, we still believe that genetic engineering will play a major role in agriculture and food production in the near future.

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