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Genetic Engineering
Herbicide Resistant Soybean

Preface

We have chosen the soybean and its resistance to herbicides as a theme for our work because it is topical. Today, human nutrition is widely discussed and lifestyles such as vegetarianism and veganism are in vogue.
We have already heard a lot about the fact that soy production is not very sustainable and that rainforests are being cleared to make more space for soy fields. On the one hand, the over-production of soya as feed for cattle and other animals is criticized, and on the other, soya products such as tofu are considered an important source of protein to replace meat and dairy products. Opinions about soy as a crop are therefore very different. Whether the genetic modification is good or bad is also very controversial. Therefore, the topic is very topical and interesting for us.
In this work, we will investigate the role of genetically modified soy in our society and whether and why it is necessary. In addition, we want to investigate how it is designed, what reasons can justify the use of genetically modified techniques and where disadvantages can be found. In order to answer these key questions, we researched the general topic, investigated a specific method of genetic engineering and conducted an interview with Hans Stettler from "Agrokommerz", an institute that supplies feed manufacturers and large-scale farmers with specific raw materials (soy is one of them). Furthermore, we will discuss the advantages and disadvantages of genetically modified (GM) soybeans.

Introduction

Most of our soy grows in North and South America [1], with Brazil, Argentina and the USA being the largest producers in the world [2].
Today there is much talk about the questionable sustainability of this plant. What many do not know is that about 75% of the soya grown worldwide is fed to our animals because it contains a lot of protein and is therefore a good source of energy and therefore an easy way to obtain many meat, egg and dairy products in a short time.
About 80% of the soy is genetically modified. The USA and Argentina in particular are concentrating on the "optimized" plant, although the EU, as one of the leading customers, was demanding more and more unmodified soya [2].
To meet the great demand for supplies, farmers try to minimize the resources and manpower required for production. The answer seems to be monocultures [3]. If only one crop is cultivated on a given area, then only one type of fertilizer is used and the amount of water and the required harvest time are the same for all plants. This leads to fewer logistical and financial complications and less time expenditure.
Herbicides are used to keep monocultures free of other plants. They attack weeds of all kinds, but also the plant itself if it is not made resistant. Herbicide resistance is achieved by genetic modification. GMO-crops have had a major impact on agriculture over the last 20 years. There are many advantages associated with this, but also major disadvantages. A widely used method for the genetic modification of crops, in particular of soybeans, is the Agrobacteria-mediated transformation method. We are concentrating on this method, more specifically on Roundup Ready soybeans, which are GM-soybeans produced with this transformation method.

Genetic Engineering Technique of Roundup Ready Soybeans

Roundup Ready Soybeans are genetically modified soybeans whose DNA is designed to resist the herbicide glyphosate. Glyphosate is a non-selective herbicide discovered in 1970 by Monsanto chemist John E. Franz. The Monsanto Company was an American agrochemical and agricultural biotechnology company that existed from 1901 to 2018. Monsanto developed Roundup, a glyphosate-based herbicide that soon became the most widely used herbicide in the US and a common herbicide used worldwide. When applied to a plant, it is absorbed by the leaves and other green parts and moves from there to the growth points of the roots and shoots of the plant. There it inhibits the plant enzyme 5-enolpyruvylshikimat-3-phosphate synthase (EPSPS) [4]. This enzyme participates in the biosynthesis of the aromatic amino acids phenylalanine, tyrosine and tryptophan via the so-called Shikimate pathway. These aromatic amino acids are essential for plants, animals and humans. Since the enzyme EPSPS is only produced by plants and micro-organisms, animals and humans must obtain the amino acids from their food. Glyphosate is a competitive inhibitor of phosphoenolpyruvate (PEP), which is the endogenous substrate molecule of the plant normally associated with EPSPS in the shikimate pathway. Since glyphosate has a similar structure to PEP, it can combine with the enzyme EPSPS. In fact, it binds even more tightly to the enzyme than the PEP substrate. By inhibiting the enzyme, it inhibits the catalysis of the enzyme and thus closes the shikimate pathway, preventing the biosynthesis of the three essential amino acids. Eventually, the plant dies from a lack of aromatic amino acids [5]. Since Roundup, the glyphosate-based herbicide, kills every plant, farmers needed a way for their plants to withstand the effects of the herbicide. Monsanto developed the first genetically modified plant seeds that could withstand Roundup and called them Roundup Ready Crops. The genetically modified Roundup Ready Soybeans were introduced to the market in 1996, followed by several Roundup Ready plants such as maize, rape, cotton and much more [6]. In the 1980s, Monsanto Company scientists discovered the glyphosate-resistant mutant EPSPS enzyme in the Agrobacterium CP4 strain on the aroA gene. (Aro denotes genes that regulate the biosynthesis of amino acids and A stands for a mutation type of the gene.) This glyphosate-tolerant form of the EPSPS enzyme is called CP4 EPSPS. It has a reduced binding affinity for glyphosate, which means that it binds to the endogenous PEP substrate of the plant and not to the competitive inhibitor glyphosate. Thus, the essential amino acids can be biosynthesized despite the glyphosate. The aroA gene can be introduced into soybeans using various transformation methods [4].

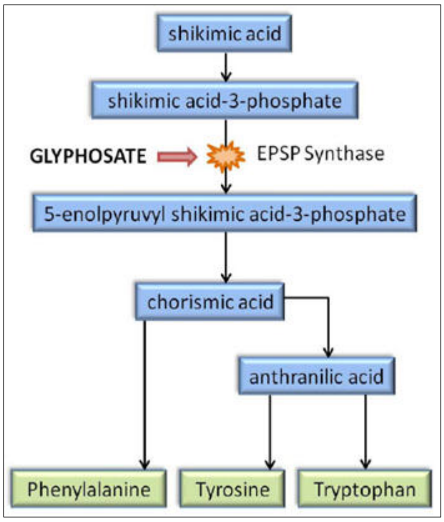

Figure 1: Glyphosate interferes with the shikimate pathway and the biosynthesis of essential amino acids

An efficient method for introducing the desired gene into the soy plant is the Agrobacterium-mediated transformation method. In this system Agrobacterium tumefaciens, the most commonly used species of the genus Agrobacterium, are used [8]. This bacterium infects wounded plants and naturally inserts one or more segments of its transferred DNA from the Ti (tumor inducing) plasmid into the genome of the host plant to force it to produce food for the growth of the bacterium. This leads to the growth of tumors and diseases such as crown gall disease. Researchers have succeeded in using A. tumefaciens as a natural genetic engineer. By isolating the Ti plasmid and replacing it with DNA containing the desired aroA gene with the CP4 EPSPS (see Figure 2)[7].

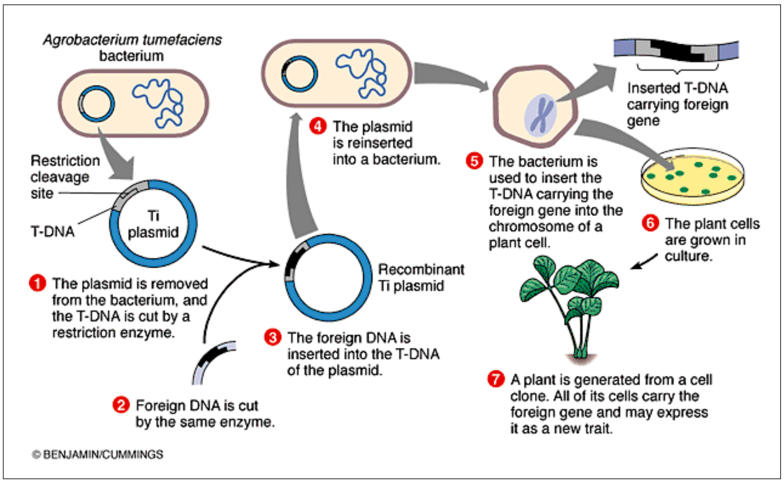

Figure 2: Agrobacterium-mediated transformation method

In the transformation process, the soy seeds are placed in a medium that contains all the elements necessary for their growth. After five days, when they have reached the so-called cotyledon stage, they are provided with small horizontal cuts (see Figure 3). Then the A. tumefaciens bacteria are poured over the cotyledons. From their wounds, the cotyledons excrete phenolic compounds that attract the A. tumefaciens which enter the host plant. After about 30 minutes, the cotyledons are placed on a filter paper containing a co-culture medium for three days (see Figure 4). During this time, the bacterium inside the plant introduces the transferred DNA into the plant cell, which it recognizes as one of its own. The cotyledons are then placed in a medium containing the chemical against which the soy should be resistant. In the case of successfully genetically modified plants, the shoots begin to grow (see Figure 5). In this way, the soybean is now immune to glyphosate and can be grown in an environment where Roundup or other glyphosate-based herbicides are used.

Figure 3: small cuts in soybean cotyledon

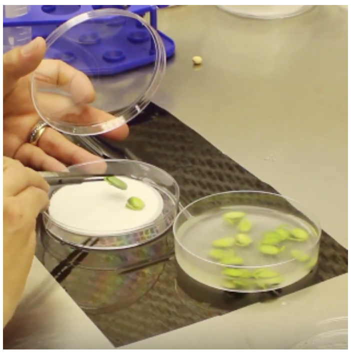

Figure 4: cotyledons are placed from a solution containing A. tumefaciens bacteria (right) to a filter paper containing co-culture media.

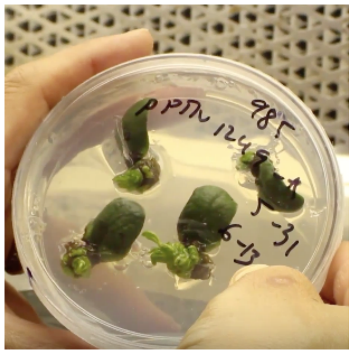

Figure 5: Clusters of new shoots are produced and the genetically modified soy plant grows.

Interview with Hans Stettler, Agrokommerz

Hans Stettler is Chairman of the Executive Board of Agrokommerz, Member of the Board of Directors and active in trading.
Agrokommerz do not sell the soybeans but soy flour (technical term: soya extraction grist (German: Sojaextraktionsschrot und Soja Kuchenmehl). We chose the company because it is a big institute that plays a role in international economy.

- Are we right with the assumption that your institute does not use genetic engineered soya?

Since many years the Swiss market only provides soy, which is non-GMO. This is consistently controlled by the GMO-analysis

- Which reasons led to your decision against genetically modified soya?

We did not decide against GMO-soya. The market, which means Swiss buyers (consumers, supermarket chains and environmental organizations) demand non-GMO raw materials. We deliver what our customers wish for.

- Which reasons might, in your opinion, speak for genetic engineering of soya?

Estimated 80% of the worldwide produced soya is probably GMO-soya today. There are many reasons for the heavy distribution of GMO-soya. GM-soya bring a better yield, the cultivation is all in all more economic. The cultivation of GMO-soya is also more ecologic because the overall usage of agrochemicals is less than in conventional ways of cultivation. Products out of GMO-soya tend to have less arrears of agrochemicals than conventionally produced soya.

- Do you think the massive spreading of genetically engineered soya (and crops in general) will someday lead to the extinction of the original plant?

In large parts of the world genetic engineering is used for the production of food. The whole producing branch worldwide stands under permanent observation by authorities, consumer- and environmental organizations such as WWF and Greenpeace. Until today there are no considerable incidents public. Because of that I answer this question with “no”, I do not think that the spreading of GMO-plants will lead to the extinction of natural crops.

- How much of the soya consumed in Switzerland (by humans and animals) is in your appraisal actually grown here?

The soya which is grown in Switzerland only covers a very small part of the total demand of our country. I assess the share to 2 to 4%. Recently the rising consumption of soya is heavily criticized, because of the clearing of huge areas of rainforest and the promotion of monocultures.

- Can the production of soya, if masses are kept moderate, be sustainable in comparison to other crops?

The question contains a wrong assertion, which I want to correct right away. Nowhere in the world rainforest is cleared to grow soy. Soya is not a primeval forest-plant and for sure not a rainforest-plant. Soya prefers steppe like landscapes.
The critics at the soya cultivation in Switzerland is stronger than anywhere else in the world. These critics are partly unobjective and infiltrated with half-truths and falsehoods. Environmental organizations such as the WWF influence the general perception by tendentious, one-sided and unobjective portrayals.
Whether we are going to consume more or less meat in future, soya will stay, in my appraisal the most important protein source on this planet. Soya has the capacity to take of the nitrogen of the surrounding air and convert it into usable proteins. Because of that soya does not need any nitrogen in form of artificial fertilizers. Soya flourishes also on barren grounds, in regions where more demanding plants as e.g. wheat would barely grow.

The cultivation of soya is relatively gentle to resources, needs little chemistry and water. From that perspective soya the right cultivated plant for many dry regions in the world.
The significance of soya for the diet of the still growing world population is central. In an overall assessment soya is in my opinion one of the most sustainable cultures of all.
Soya is well suitable for biological farming because, as said above, it brings good yields without artificial fertilizers. The organic agriculture was probably the most sustainable form of cultivation but the yields are smaller and the products more expensive. Because of that, bio-soya is probably going to stay a barely distributed product.

*The interview was translated from German into English.

In addition to the interview, we have asked for some photographs of the laboratory work. Since Agrokommerz does not have an own laboratory, Mr. Settler forwarded us to Dr. Diana Hormisch, the Head of Microbiology, Food and Agro Diagnostics from BIOLYTIX AG, with whom his institute collaborates. BIOLYTIX AG is a service company specializing in gene expression as well as microbiological and molecular water, feed and food analysis. The laboratory in Witterswil sent us following pictures of their work in

Figure 6: Soybeans on the plant

Figure 7: Samples in the laboratory before grinding: 1) soybeans (left) and 2) soycake as feed, pelleted (right); in the background grinding bowl and balance

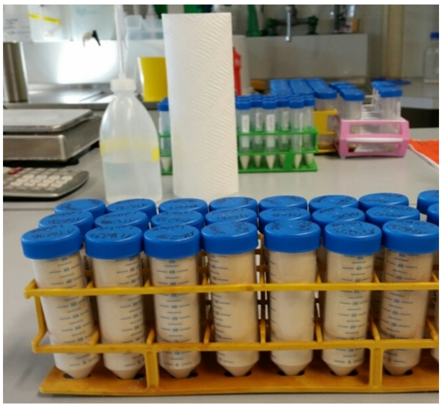

Figure 8: Sample material after grinding, filled into sample tubes;
in the background: tubes for weighing the sample quantity for DNA extraction from the ground sample

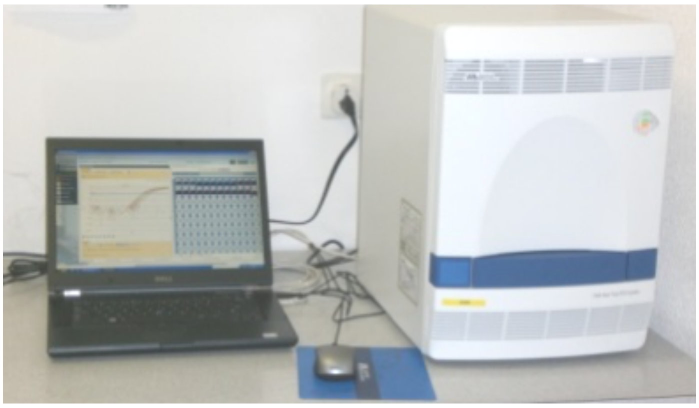

Figure 9: real-time PCR device for DNA analysis of genetically modified soybeans

Discussion

Progress of the Roundup Ready Soybean

The genetically modified soybean was one of the first GM-crops introduced for commercial use and is the world's largest GM-crop in terms of area. Monsanto Company was the first company to introduce GM-soy and it was a big step in GMO research. In recent decades, the majority of research laboratories have made efforts to improve the genetic modification of plants. Roundup Ready Soybeans were the first and very widely used genetically modified soybeans. In 2005, 87% of US soybean fields were planted with glyphosate-resistant varieties. With the increase in herbicide-resistant soy, glyphosate levels, especially Roundup, per hectare increased as shown in the first graph in figure 10. This is also due to the fall in glyphosate prices over the last 20 years, as can be seen in the second graph. The increasing use of herbicides has led to some controversy. In particular, the potential health risks posed by Roundup, Monsanto's famous herbicide, have led to numerous discussions. Only recently, Trump administration decided to keep Roundup on the US market and insisted on its safety for humans despite the thousands of lawsuits filed by people who claimed they had given them cancer. The Environmental Protection Agency (EPA) states that glyphosate does not cause any health problems when used according to instruction labels [9]. Because glyphosate was easier to use, less of the other herbicides available were used. Many of them were more present in the runoff water and are more harmful to the environment than glyphosate.

A major concern with genetically modified plants such as Roundup Ready is that weeds and other plants also develop resistance to herbicides. The third graph in Figure 10 shows the increase in resistant weeds. This can only be prevented if farmers rotate Roundup Ready crops with other crops and use alternative herbicides. By 2016, there were almost 30 different types of glyphosate-resistant weeds worldwide [10].

Agrobacterium-mediated transformation is the most commonly used method of plant genetics. The system is constantly being improved and successfully implemented. There will be further research and progress in these methods as the demand for soy increases with the world's population growing and the soybean gaining in importance.

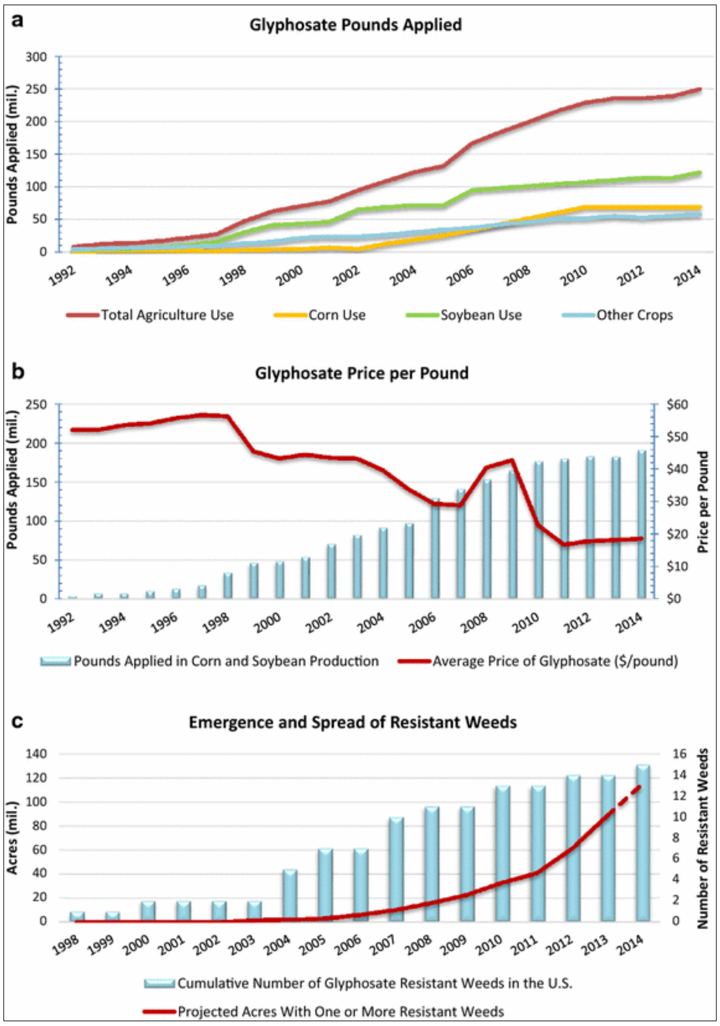

Figure 10: Graphs that show the amount of glyphosate usage and price and the spread of resistant weeds from 1992 to 2014

Ethical Aspects

Because demand for soy is so high, farmers have no choice but to work with monocultures to produce effectively. The risk of diseases and other plants growing between the desired crops in monocultures is very high. Herbicides ensure that there is only one plant in this area. The resistance of plants to herbicides is therefore an important factor that makes monocultures very safe and lucrative. The world population can be supplied with a lot of food, and farmers do not have to fear poverty because they can easily live from these fields.
If we also want an alternative to meat, genetically modified soy is the most effective. Non-GM-soybeans would not give us as high a yield as GM-soybeans. Since climate change is the greatest threat to our existence on this planet, a more environmentally friendly option is needed.

A major concern about herbicide-resistant soy is that the soil and all surrounding plants are still suffering from herbicides, which can endanger biodiversity and make the soil infertile. The lack of natural diversity in the fields also makes the crop more contestable, which is a general problem of monocultures.
As already mentioned and shown in Figure 10, the amount of glyphosate in the fields has increased sharply and more farmers are exposed to it. Since it is still unclear whether this exposure causes health problems, there is much controversy about herbicides, especially Roundup.
Another disadvantage of GM-crops is that they can "outcross" or "cross-pollinate" non-GM-crops by wind distribution or pollinators such as insects or birds. This has serious consequences for farmers who are victims of this contamination. Organic farmers can lose their organic certification, which leads to enormous economic losses. In addition, farmers were sued for "seed piracy" because unauthorized GMO-crops appeared on their fields.
Another problem with herbicide-resistant soy is the aforementioned resistant weeds. Farmers affected by such resistant pests must use older and more toxic chemicals or do more work, which is of no benefit to their economic gain, health or the environment [10].

Summary

With the world's growing population and the associated increasing demand for fast and lucrative methods of food supply, soya will play a major role in our diet in the future. There is no other way for farmers to meet these requirements than to ensure that they can produce enough and that their plants are protected from herbicides. The most effective way to protect the plant to date is genetic modification. As with soybeans, the discussed Agrobacterium-mediated transformation method is applied. GM-soy brings many benefits to the economy and makes agriculture much easier. On the other hand, there are disadvantages such as herbicide-resistant weeds and controversies about the toxicity of GM-soy. Considering that the first genetically modified soybean was introduced only 21 years ago, we are still at the beginning of perfecting the techniques of genetically modified food. Personally, we note that soy has the potential to be a great alternative to meat, especially as at the moment most of the culture is used as animal feed anyway, which would not be necessary if we were to make soy a more direct part of our diet.

Sources

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