Applications of Genetic Engineering and Bio-Technologies

Gene Manipulation in Tomatoes – „Flavr Savr"

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1. PREFACE

„Applications of Genetic engineering and Bio-Technologies“ was the main topic of this year's semester work. We wanted to specialize in the gene manipulated corn or rice as it is the main food source for many people in the world, but these two topics were already taken from other groups. Then we thought of treating gene manipulated vegetables in general but we had to narrow the topic into „Gene manipulated tomatoes“, even though we know that in biological terms the tomato is a berry.

We use tomatoes in our daily lives and it's a very common vegetable in our food. Tomatoes were the first genetically modified foods to come on the market and today they are no longer cultivated. Tomato fruits are particularly rich of nutritional compounds such as Vitamin C for example. In the last years, tomato consumption has further increased since it was demonstrated that tomato fruit could protect against diseases, such as cancer and heart diseases.

These were our questions when we were thinking about our chosen topic:

1. What is the Flavr Savr tomato?
2. Why was the Flavr Savr tomato the first gene manipulated vegetable on the market?
3. Which methods of gene engineering are applied?
4. Why is especially the agrobacterium tumefaciens method used in tomatoes?
5. Which qualities of the tomatoes were improved due to gene engineering?
6. Comparison between insecticides and gene transformation
   → Advantages/Disadvantages?
7. Are gene manipulated tomatoes harmful for human beings?

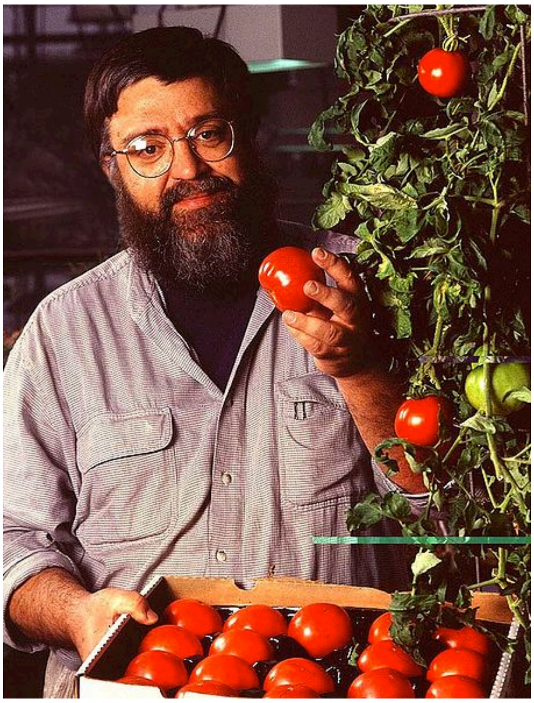


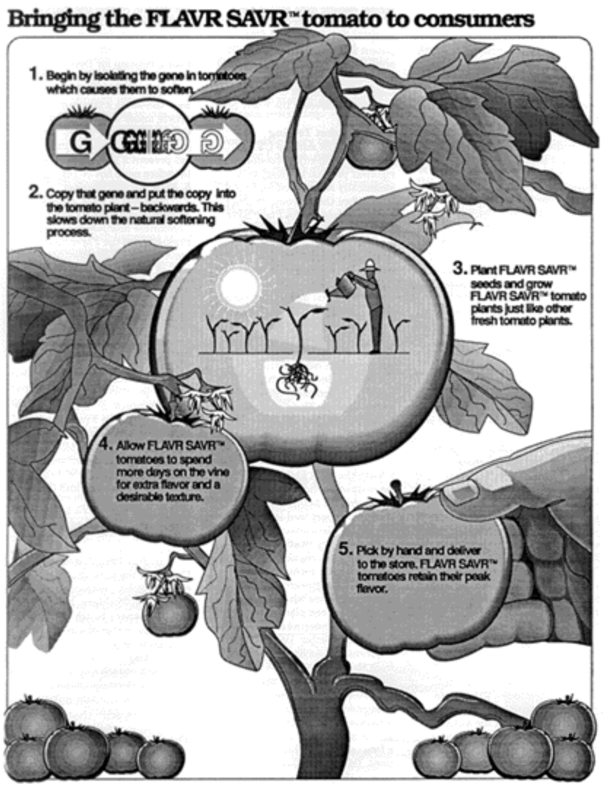
Figure 1: A plant physiologist with examples of bioengineered tomatoes

2. Introduction

2.1 Context and Scientific History

Tomatoes are very common and used in our everyday food such as in ketchup, tomato sauce, tomato paste, drinks (juice) and making our food a lot tastier. It is originated in South America and was brought to Europe due to the Spanish colonization of the Americas in the 16th century. After centuries of breeding there are thousands of varieties grown worldwide. Today, the tomato is one of the most important vegetable crop worldwide with a total production of around 141 million tons on a cultivated area of around 5 million hectares. In the late 1980s Agrobacterium-mediated genetic engineering techniques were developed and could successfully transfer genetic material into the nuclear genome of tomatoes. The first edible fruit where this was possible were the tomatoes. This was the case because it was easy to pass through the thin cell wall of the tomato and transfer the genes. Moreover they have a short generation time and that's why it was suitable for the research on genetic engineering to understand the nature better. Then the researchers realized that you could improve the fruit qualities with genetic engineering. In 1987 the researchers of the Californian company “Calgene” identified the polygalacturonase (PG) gene which was responsible for the softness of the tomatoes, developed the methods of transformation and regeneration and produced the tomato plant with an antisense gene which prevented the formation of PG. After many field trials the U.S. Department for Agriculture gave permission in 1992 to bring the transformed tomatoes to the market. In May 1994 the “Calgene” members gained experience in the conventional fresh-market tomato business and met with community leaders, media representatives and consumers in Davis and Chicago where they introduced the FLAVR SAVR tomato. Because of high production and distribution costs it was not profitable even though there was a high demand. The sales of this product however declined dramatically in 1998. Customers were not sure anymore about buying the genetically engineered food and slowly the tomato was removed from the market

Figure 2: Flavr Savr to attract consumers in 1994



2.2 Usage of the technique

Genetic manipulation

Agrobacterium-mediated transformation has been used to manipulate tomatoes since 1980s. The transformation efficiencies obtained in various cultivars range from 10 to 41%. Many factors were believed to be important for tomato transformation using Agrobacterium tumefaciens, including the application of nurse cells or acetosyringone to the culture or pre-culture media, the type of explants, the Agrobacterium strain used and its concentration, etc.

There are two other methods, which can be used to manipulate tomatoes:

1. Direct gene transfer through PEG-method or electroporation
2. Biolistic gene transfer

Both have a disadvantage in contrast to gene transfer through Agrobacterium tumefaciens. Both are not as specific as the Agrobacterium-mediated one is, it's natural and also cheaper than other methods.

Fruit quality engineering

Tomato fruit quality includes several aspects that may be divided into two groups: *organoleptic properties* and *nutritious contents*. Organoleptic quality involves color and texture of the fruit, but also taste and aroma, whereas nutritional quality refers to the content of metabolites contributing to the intake of nutritious such as sugars, carotenoids, flavonoids, ascorbic acid and folate.

Many quality traits show a variation and contain the joint of many genes. The traits are also induced by environmental conditions. Although they have a complex inheritance, fruit quality traits have often been engineered in tomato through genetic transformation. Controlling of single major genes took place, which is involved in the regulation of a desirable phenotype. In addition, genetic transformation has often been successful in improving fruit quality-related traits in tomato.

In general, there are three main goals of engineering strategies in plants:

1. The improvement of a desired trait
2. The decrease in the expression of a specific unwanted trait
3. The development of a novel trait (i.e. a molecule that is produced in nature but not usually in the host plant, or a completely novel compound).

Flavr Savr1[1]

By natural ripening, tomatoes get softer as the enzyme polygalacturonase (PG) is able to dissolve the plant's cell-wall pectin. Because of its softening, it doesn't stay fresh any longer and it makes tomatoes more susceptible to being damaged by fugal infections.
Scientist wanted to slow the ripening process of the tomato and thus prevent it from softening through genetic engineering and they also wanted that the tomatoes have its natural color and flavor. Researchers at the Californian company Calgene proposed to introduce an antisense gene in Flavr Savr tomatoes to suppress PG accumulation in ripening tomatoes. Antisense genes reduce the formation of PG. The genetically changed tomatoes are plucked before ripened (green) and are then purged by ethylene gas to ripe them artificially. This process allows for easier handling (transporting) and extended self-life. Flavr Savr tomatoes could ripe on vine, without decreasing in their self-life, because the process of slowing down the softening of Flavr Savr tomatoes allows the vine-ripe fruits to be picked like green tomatoes without any damage to itself.

Agrobacterium tumefaciens

Agrobacterium tumefaciens is a soil borne pathogen responsible for crown gall disease affecting many higher species of plants. Economically, the A. tumefaciens is a pathogen of walnuts, grape vines, stone fruits, nut tree, sugar beet, horse radish and rhubarb. It is able to transfer its DNA to plant cells naturally, that's why it is used in genetic engineering to transfer the genes to plant cells for an improvement. But this method is used only for dicots, that's why this method cannot be used for crops and corn for example (which are monocots).

Figure 3: Agrobacterium tumefaciens

[1] Flavr Savr in the whole script refers to Flavr Savr™

3. Engineering technique

3.1 Explanation of the Agrobacterium tumefaciens

As it is already known that enzyme polygalacturonase (PG) is concerned for softening of tomatoes. In the Flavr Savr or "anti-mud" tomato, polygalacturonase are formed only in very small quantities. For the genetic engineering, the gene for polygalacturonase was isolated from the tomato and placed in the reverse direction ("antisense") as the natural gene behind a plant promoter.

This gene is transferred into tomatoes through the agrobacterium tumefaciens (transfer vector). Agrobacteria are natural plant parasites, and their natural ability to transfer genes is used for the transforming antisense gene into Flavr Savr tomatoes.

But when it transfers its gene into tomatoes to create a suitable environment for itself and leads to the crown gall of tomato cells.

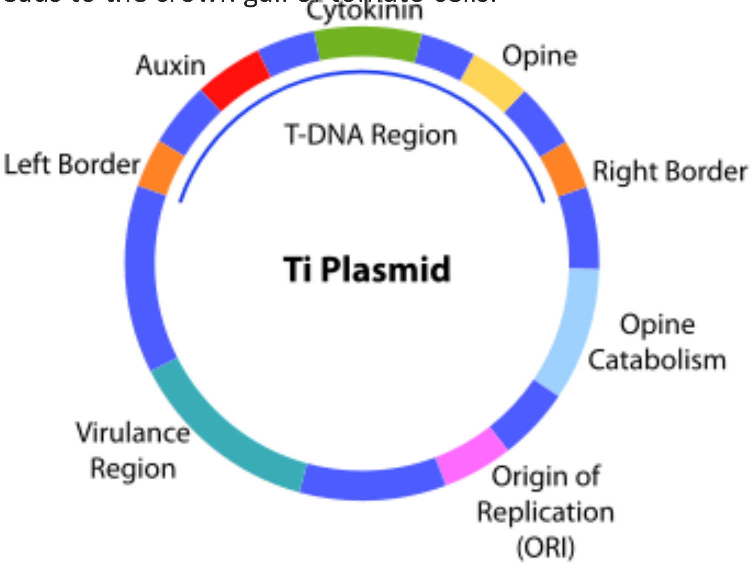
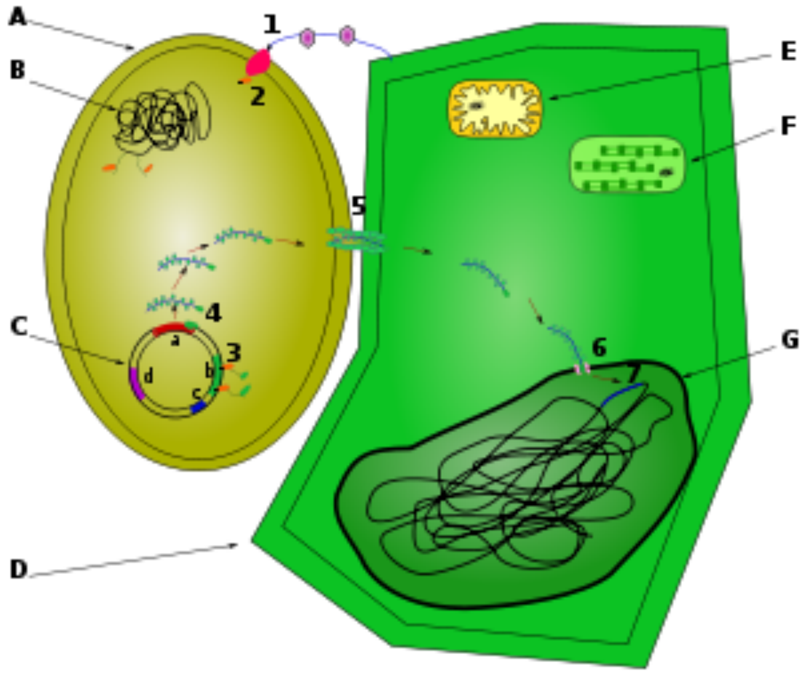


Figure 4: T-DNA of A. tumefaciens

Its Ti plasmid is responsible for this tumor, so for genetic engineering T-DNA is removed from the bacterial plasmid and replaced with antisense gene. The easiest art to transfer the gene is the infection of the tomato's plant leaves. The plant leaves are dived into the liquid culture of Agrobacterium tumefaciens, then the excess liquid is removed and the plant leaves are put with their bottom part on the upper side on the special nutrition tile. There they stay for 2-4 days to give the Agrobacteria enough time to transfer the T-DNA into the injured part of this tissue. Now Flavr Savr cells contain antisense gene.



A: Agrobacterium tumefaciens

B: Agrobacterium genome

C: Ti Plasmid:
a: T-DNA, b: Vir genes,
c: Replication origin, d:
Opines catabolism genes

D: Plant cell

E: Mitochondria

F: Chloroplast

G: Nucleus

After transformation in a specific ripening process both genes are turned on, that means transcribed into mRNA. The natural single-stranded mRNA and the genetically made single-stranded antisense mRNA form a duplex because the two strands of the DNA fit together. This “interception” of the natural mRNA prevents the formation of polygalacturonase. The cell walls remain intact and the tomato is more plump and round.

Figure 5

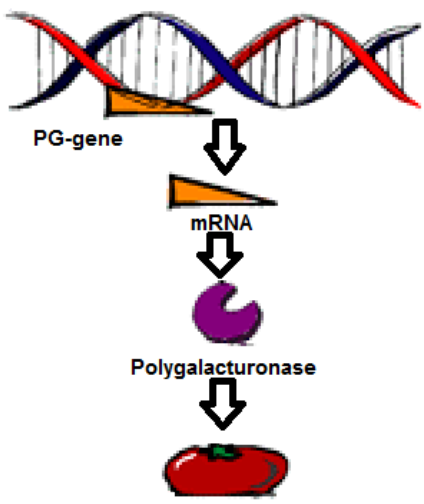


Figure 6

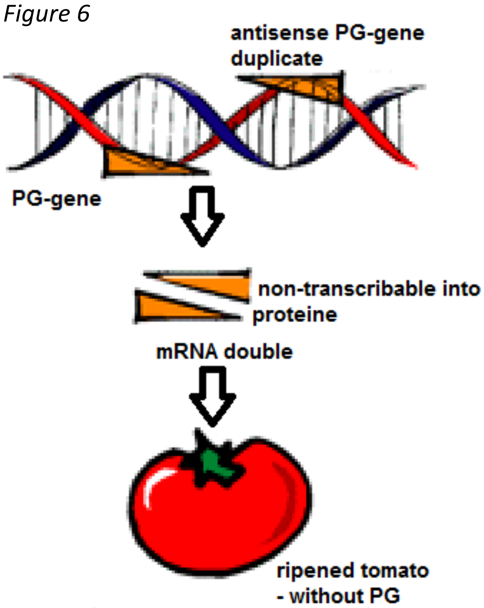


Figure 5: Without antisense gene and the formation of polygalacturonase → soft
Figure 6: With antisense gene → it gets hard

Genetically changed plant cells contain also a marker gene, which helps to select the transformed cells from the non-transformed ones. Marker genes are resistant to chemicals such as antibiotics or herbicides. After the transformation, all the plant cells are put into the antibiotics liquid. Only transformed cells survive as they contain resistance to antibiotics. Then the Flavr Savr cells create a tissue culture and the transformed plant regrows from its transformed cell's tissue culture. Now the Flavr Savr tomato plants are genetically transformed.

4. Interview

Interviewed Person: Dr. rer. nat. Jan Lucht, biologist (biotech, nutrition and consumption) at Internutrition (Scienceindustries)
Date: 4/10/12, Basel

1. Hello Mr. Lucht and thank you for being our interview partner. Please introduce yourself.

My name is Jan Lucht, I'm a biologist, I've studied biology, worked for a long time in the basic research as a microbiologist and already used the gene technology as a method there. Then, I've worked several years in the section of plant research and also with gene manipulated plants even though my intention was never to develop beneficial plants but it was nice to understand how plants work with the basic research. There I've also used gene technology several years and routinely as a method and I've stopped with the practical research in the lab in 2003 and went to a trade association. I'm still working there in the section of public relations, gene technology, agriculture and nutrition. Meanwhile the name of the trade association has changed to Scienceindustries and is now the chemistry, pharmaceutical, and biotechnological companies in Switzerland. It has now 250 member companies like the big companies in Basel (i.e. Nestlé) and is therefore not only a chemistry but it's a larger field.

2. What does Internutrition deal with?

Internutrition used to be an independent association. I've started my work there in 2003 and was the general manager until 2010. In 2011 this small association was integrated into the bigger union but my activity is still the same, even the office is in the same place and now I'm employed at Scienceindustries. In Scienceindustries there's a working section which is called “Biotechnology - Nutrition and Consumption” but deals also with nanotechnology for food production, pesticides and with residues in food which is an important aspect for the consumers for example. I'm a specialist in the section of “Gene Manipulated Plants”.

3. What do you personally think is interesting about gene technology?

I've started studying biology in Konstanz (Germany) and found it very exciting because you could do a lot of things with it. In 1985 I went to the U.S. as an exchange student and could do practical work in the lab for the first time. I've been given a short research work which was about metabolic processes in yeast. Gene technology was used there as a method to turn on or turn off specific processes. In the lectures I've heard that there are such methods used but they weren't common in Europe. Whereas in the U.S. they were standard methods. That was very fascinating for me what you could do with that and it was very useful. At that time gene technology was just used as a tool to understand the nature.

4. What was so special about the tomatoes since they were brought to the market being the first gene manipulated fruits?

To manipulate plants genetically is not as easy as with bacteria or yeast for example. Big problems occurred because the plants have a thick cell wall. To change the cell you have to get through the cell wall. There are plants which are more easy and plants which are more difficult to get through the cell wall. With crop and corn it's much more difficult to do so and that's why there have been several years until you could change them genetically. Tobacco is even easier and was the first gene manipulated plant. It wasn't genetically changed in order to gather something but it was just easy because of technical reasons.

5. Are the genetically changed tomatoes still on the market today? We've read somewhere that they weren't available on the market anymore. Is that true?

That's right. They weren't as good as the researchers thought they would. You've probably read about this Flavr Savr tomato where the natural ripening process is interrupted because when fruits and plants stay in the sun, they get redder (tomatoes), tastier and softer. But like this you can't transport them. Because of that, their natural ripening process is interrupted. When the tomatoes are green and hard, then you can transport them better and add a gas, ethylene, so they can't be broken during the transportation. With the help of that gas which is a sign for the tomatoes to ripen they get nicely red and look good. But the problem was that their aroma wasn't fully developed. So the researchers looked for solutions and found an enzyme which is responsible for the softness of the tomato. They could turn off this enzyme so that the tomato could stay hard. In the end the tomato was red, tasty and hard. After a few years, the people buying these tomatoes (which were also more expensive than the non-gene manipulated tomatoes) weren't sure anymore about this gene manipulated tomatoes and thought that they could be harmful. In order not to cause any protest actions, the producers removed these tomatoes from the market.

6. Is there any other existing method except of the one with the bacteria agrobacterium tumefaciens to transfer the traits into a tomato?

If you want to genetically change a plant then you have two main methods:
The agrobacterium tumefaciens is the first one and mostly used because these bacteria occur in nature and are not man made. They're widespread in the soil, are pathogen of plant diseases, can be transferred into cells of plants by itself and can change plants genetically. With that the plants make root crown tumor and produce substances which the bacteria can eat. It's relatively easy to change bacteria genetically because you can make the membrane permeable by using chemicals. Then you can add traits which the bacteria do gather, so they're genetically changed. This works very good in the tomatoes, too. The biggest problem was how to come through the cell wall. The agrobacterium could do this.
The second method which works good as well is the direct gene transfer, where you shoot microscopic small bullets which are made from gold (or other heavy metals) and which contain the DNA, into the cells. The first attempts in Switzerland were made with shotguns with gunpowder. Meanwhile today it is done with gas pressure.
There are some other methods which you can use but they're technically lavish and are rarely used.

7.Can these gene manipulated tomatoes be harmful? How do people react concerning these food products?

I don't think so. But I think that it depends on the traits you want to bring into the tomato. There have been two genes transferred into the tomato. The one was for the softness of the tomatoes. I can't imagine that it could fulfill another biological function than the one it is

responsible for. There has been a second gene indicated which was the marker gene to find the cell which had incorporated the external DNA. And this works well with the agrobacterium. It's been discussed wether it was harmful if you transfer antibiotic resistance genes into food. Some people were afraid that when you eat a food product that some bacteria which you already have naturally existing in your body would pick up this gene and become resistant to antibiotics. This means that pathogen would become resistant to the antibiotic and you wouldn't be able to treat several diseases anymore which are actually easily controlled with antibiotics. Many scientist have dealt with this whether this is a problem and they have come to the conclusion that there is no risk. The antibiotic resistance genes are not invented by human in labs but they come from the nature. There is also no evidence for any problems. There has been no incidents that humans or animals have gotten sick because of that yet.
Most people are insecure because they don't know what it means when a plant is “genetically manipulated”. Nutrition is very important to human and it's associated with naturalness and something which you don't want to get genetically changed. However, they maybe have heard of risks or so. But the consumers in Europe are sceptical and rather cautious. Whereas in other parts of the world like the U.S . people don't have any problem with that. In Asia it's also more accepted than here. But it is also reasonable because people here can buy everything and nobody goes hungry. Everything works how it should be so why do we need new technologies? So there's no concern in changing anything.

8.Which are the advantages and disadvantages of gene technology compared to insecticides?

I think that it's an advantages that it is much more specifically, that means you can make insecticide resistant plants. Bt-corn is an example which is widely grown and can protect itself against insects. The advantage is that this Bt-protein is directed only to specific insect groups, in this case the corn borer, so it doesn't concern all insects. It doesn't work with bees, earthworms other insects which are in the fields. However, it could have an effect on butterflies but the cornfields are very unattractive for them because the corn doesn't flourish and doesn't produce any nectar. It has advantages for lots of insects in the fields because the option of the insect resistant corn is that you splash the fields with broadband insecticide. This concerns bees and other insects too which are in the fields and concerns not only the corn borer. There has been a considerable count on all the insects which lived in the fields and which then have been investigated. Then it was clear that the fields with the least diversity were the conventional fields which have been treated with insecticides and from which all insects had suffered. Much better however were the fields with the Bt corn which haven't been treated with insecticides. There, there were less pests and less spiders which the corn borer had been feeding itself from. Since there were less corn borers and the number of spiders decreased naturally. The fields which showed the least diversity were the ones farmed biologically. There were lots of beneficial organisms as well as pests where the yield was less. The biological farming is not convenient for countries which want great yields. The yield depends on the amount of money you want to invest, and this you have to decide on your own.

Notes: We've interviewed Mr. Lucht in German so we had to translate the whole interview into English language.

5. Discussion

5.1 Progress made with agrobacterium tumefaciens

The transgenic tomatoes can ripen on the vine and have not to be picked green. They can develop in addition to the color, taste and valuable food ingredients (e.g. vitamins), although they are not soft. They can be transported on long distances as visually attractive and flavorful tomatoes. This leads to more global trade and has a great impact on agriculture. Longer storage times are possible without refrigeration. But the tomatoes can lose their vitamins during transportation and are then therefore not so tasty anymore. They were also more expensive than the non-genetically changed tomatoes and that's why they got less and less interesting for the consumers. Therefore the tomato was taken away from the market.

At the moment scientists are developing new traits in tomatoes like increased resistance to pests, environmental stresses, health benefits or being more nutritious.

5.2 Advantages and disadvantages of the used technique

PRO	CONTRA
Environment and plants protection without chemicals	More expensive than insecticides → developing countries cannot effort the money for gene manipulated food → they use therefore more insecticides
Improvement in plants' qualities	Tomatoes lose their aroma
Economically beneficial	Ring of nature chain → insects may be influenced → can develop resistant mechanism
Specific application → you can choose any trait you want to transfer into the plant's cell	Consuming foods which were made antibiotic resistant may give the same property to human → failure of antibiotic-based treatments
Progress in science	Beneficial foods that carry toxic effect genes can cause human illness

6. Summary

The first genetically engineered fruit brought to the market in 1994 was the tomato, called the FLAVR SAVR. In the beginning, it gained a lot of attention from the people because it promised to be long-lasting in freshness. But because of the big costs for the production of this tomato and the high demand, the companies producing these tomatoes had no profit. In 1998 the consumers stopped buying this product and so it was removed from the market again. However, today scientists are still developing new beneficial traits in tomatoes. The method used to transfer the genes into the cells of the tomato was the Agrobacterium-mediated genetic engineering (A. tumefaciens). The transferred genes were responsible for preventing the formation of the polygalacturonase (PG) which was relevant for the softness of the tomato. The goal was to develop a naturally red, tasty and hard tomato. There were also other methods to achieve this goal but these were more expensive and not as specific as the A. tumefaciens method. This method was very specific and does not harm the environment in contrast to the insecticides. But it is much more expensive than insecticides and is therefore not used in developing countries for example. In Europe people are not too sure about the gene manipulated products and today there are no such products in Europe, whereas in the U.S. they're very common and accepted. The tomatoes were probably a big initializing cause for the scientists to investigate other food products like crop and corn (Bt corn).

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