GM CARROTS

Genetically modified carrots may help prevent osteoporosis

EXPOSEE

A carrot helping people to absorb more calcium has been produced.

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Contents

1. Preface

Shortly after I had started my research on a possible subject for this paper, my choice fell on the genetic modification of carrots, which may help prevent osteoporosis.
My motivation for working on this topic came from my interest in osteoporosis, its causes and possible preventive measures.
I am very interested in the way genetically modified foods can be used to help prevent such diseases and how the applied techniques work.
Especially interesting to me is how genetic modifications may influence the nutritional values of food. Also, I am particularly interested in the acceptance of such genetically modified foods in our society.

Key Questions

For my research I posed the following key questions:

1. What technique is used to introduce the isolated genetic material into the carrot?
2. Where and how is the calcium stored in the carrot?
3. Can people absorb calcium provided by the modified carrots?
4. How significant could this GM food be in the prevention of osteoporosis and for people's health?

1. Introduction

To put the chosen topic in a context, one must first look at the purpose for this type of genetically modified food.
The International Osteoporosis Foundation estimates that worldwide 1 in 3 women and 1 in 5 men over the age of 50 years will experience osteoporotic fractures in their lifetime.
Osteoporosis is a bone degenerative disease, through which the bone loses its stability because of a loss of density (Sözen, 2017).

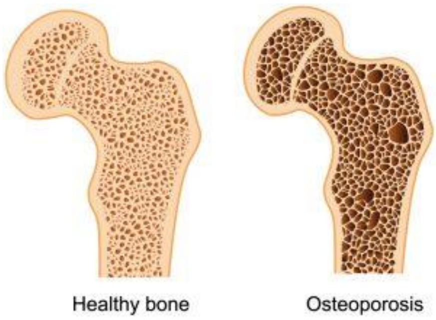


Figure 1: Healthy and osteoporotic bone, with a lower bone density.

Beside some factors which cannot be modified, such as age, gender or genetic predisposition, other factors such as calcium intake are of high relevance.
This is where this study fits in. Through genetic modification an attempt was made to increase the calcium content in carrots, with the aim of supplying people with more calcium through vegetables.
For this process, foreign genes from the plant *Arabidopsis thaliana* were inserted into carrot cells with the help of *Agrobacterium tumefaciens*. Agrobacterium was first isolated from plant tumors in 1897 by Fridiano Cavara in Napoles, Italy (Kado, 2014).
In 1983, scientists from the Max-Planck Institute located in Cologne succeeded in using *Agrobacterium tumefaciens* as a vector to introduce foreign genes into plants.

This method is already being frequently used in agriculture. Today, 30% of corn production occurs on the basis of genetic modification. With cotton, the estimated percentages are as high as 80%. This, because through genetic modification plants can be made resistant against insects, pesticides or other threats (101jahre-biotech, 2019).

Regarding osteoporosis, pharmacological treatments have been developed.
Nowadays, people affected by Osteoporosis usually receive Bisphosphonates which attach themselves to the bones and inhibit the activity of the osteoclasts, thereby reducing bone loss (pharmawiki, 2019).
However, the goal pursued by the endeavors, described in this paper is rather the prevention than the cure of the disease.
Sadly, this research on genetically modified carrots was carried out in 2008. As one will further verify throughout this paper, people are extremely skeptical about genetically modified foods and therefore do not wish to eat them. Still, the research made is a perfect example of the application of genetic modifications.

2. Description of engineering techniques

The sCAX1 antiporter

According to Dr. PhD. Kendall Hirschi, who conducted the study on genetically modified carrots, it is suspected that calcium (Ca^{2+}) is stored in the cell's vacuoles. For a long time, it had not been known which transporters were responsible for calcium uptake.
In 1996, a cDNA (complementary DNA) sequence encoding the sCAX1 antiporter protein was found by Dr. PhD. Hirschi and his team. This protein stems from the plant *Arabidopsis thaliana* (see Picture 1) and is found in the membrane enveloping cell vacuoles.
It was discovered because of its ability to suppress yeast mutants defective in vacuolar calcium transport. The sCAX1 antiporter pumps hydrogen ions out of the vacuole in exchange for calcium (Jeng, 2008).
The gene which codes for this protein is hereafter referred to as sCAX1 gene. A longer cDNA (see Figure 2) containing a N1-36 terminus was subsequently described by scientists, when they first identified the gene. This long form (lCAX1) appears to be autoinhibitory, which means that it is not able to suppress yeast mutants defective in vacuolar calcium transport. Therefore it is not well suited to their transgenic studies (Park, 2004)(Pittmann, 2001).
If the activity of these specific antiporters is enhanced, the plant could store more calcium in its vacuoles.

Picture 1: Arabidopsis thaliana

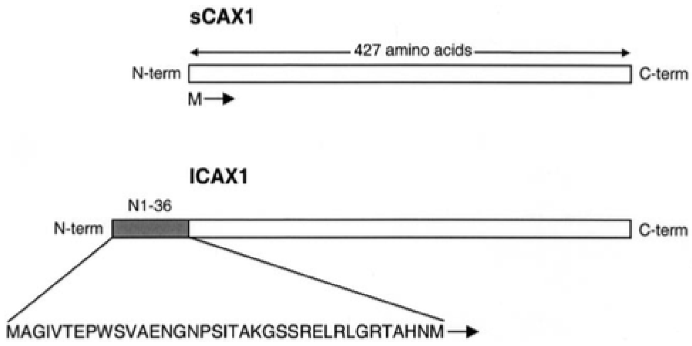
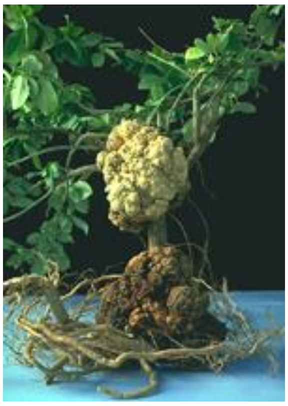


Figure 2: Structure of lCAX1 and sCAX1; lCAX1 contains 36 additional amino acids at the N terminus, the sequence of the first 37 amino acids is shown on the N1-terminus

Agrobacterium tumefaciens as a gene transporter

To insert the sCAX1 gene into the carrot they used *Agrobacterium tumefaciens.* The bacterium is found in cultivated and uncultivated ground. It can cause excrescences, a type of abnormal cancer like growth on plant roots, also referred to as crown gall disease (see picture 2). The bacterium is able to introduce the genetic information for crown gall disease into the plant's genome, if it is able to enter the plant through a wound. Because of its ability to change plant's genes, it is used as a vector to introduce specific genes into plants (pflanzenforschung, 2010).



Picture 2: Plant infested with crown gall disease

Construction of Agrobacterium tumefaciens

A. tumefaciens contains two types of genetic information, the bacterium's own DNA, and a loop DNA Ti-Plasmid (tumor-inducing Plasmid). The genetic information found on the Ti-Plasmid is introduced into the plant's DNA and then decoded by the plant itself. This causes crown galls, in which fluid is produced, which the bacterium needs for growth, but cannot produce by itself (see Picture 2) (pflanzenforschung, 2010).

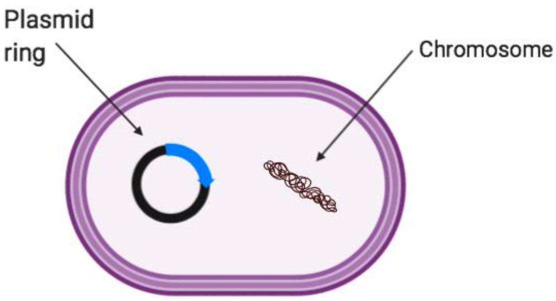


Figure 3: Basic structure of *A. tumefaciens*

Mode of Operation of Agrobacterium tumefaciens

Through a protein called VirA on its envelope, the bacterium can detect phenolic compounds, released by the wounded plant. If phenolic compounds bind to the protein VirA, another protein called VirG is activated through phosphorylation. The VirG protein activates Vir genes, located on the Ti-plasmid. Following transcription the products VirD1 and VirD2 are released. These proteins are endonucleases and stick to the Ti-plasmid to produce a single stranded t-DNA, which sticks to the VirD2 protein. This protein leads the t-DNA into the plant's cell, where it is integrated into the plant's genome (Abbott, 2018).

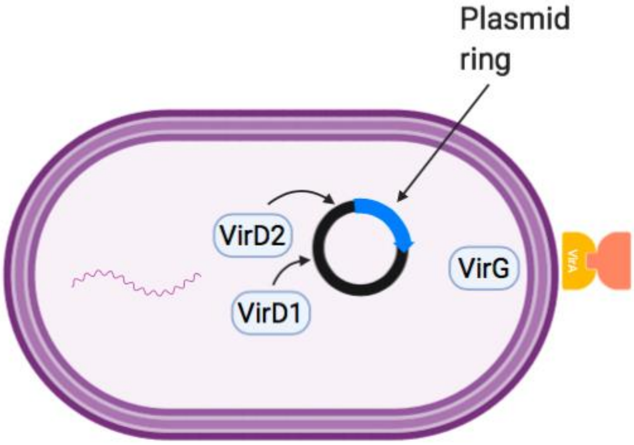


Figure 4: A. tumefaciens, mode of operation Components

Genetic transformation; technique to insert sCAX1 gene into plasmid

Inserting foreign DNA into a Ti-plasmid would often make it too long to be effectively transferred. Therefore binary vectors are used to induce the desired strand of DNA into the plant's cells. First the t-DNA containing the information for the construction of crown galls and its right and left borders are cut out with the help of enzymes. The remaining part only contains the genetic information which is needed to induce the t-DNA into the plant. This Ti-plasmid is also referred to as helper plasmid.

As a second step, the DNA-strand to be transferred is introduced into a smaller Ti-plasmid and is bonded on either side by a right and left border respectively. An antibiotic resistance gene is also present in the smaller Ti-plasmid. Only *A. tumefaciens* cells with both plasmids will grow on plants containing an appropriate antibiotic. This vector is multiplied in an E-coli bacterium and then induced into *A. tumefaciens* (pflanzenforschung, 2010).

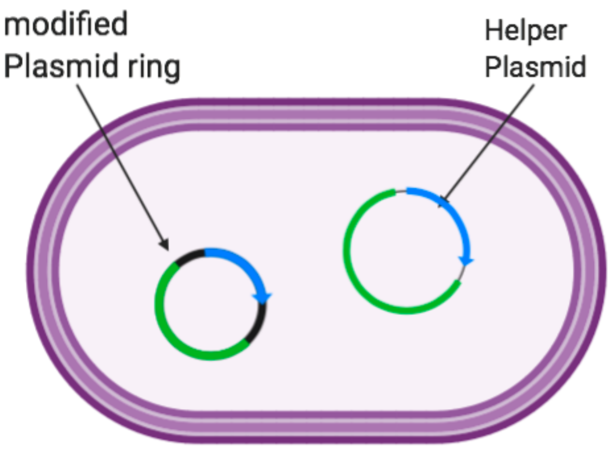


Figure 5: Modified A. tumefaciens

Cultivation of modified *Agrobacterium tumefaciens* in carrots

With the aid of the helper plasmid, the plasmid containing the sCAX1 gene can transfer the inserted t-DNA into the carrots, from which new carrot cells can be cultivated, containing the desired sCAX1 gene. According to Dr. PhD. Hirschi, it takes two years for the carrots to regenerate and stabilize their calcium level.

3. Interview with Dr. PhD. Kendall Hirschi

Dr. PhD. Kendall Hirschi led the research on modified carrots at the USDA-ARS Children's Nutrition Research Center (CNRC) in Houston, Texas. His professional interest is the nutritional improvement of agriculturally important crops. He currently works at the Baylor College of Medicine. The Interview can be found in the attachment. Information from this Interview was used in other segments of this paper.

4. Discussion

Testing and Results

To test the bioavailability of calcium in the modified carrots, a test group of 30 people (15 men, 15 women, both of different ethnical backgrounds) were fed single meals containing either modified or regular carrots. Both portions contained the same amount of calcium, in order for the absorption to be comparable. Also, milk was used to compare calcium absorption because of its high calcium content. Urine samples were taken after 1 day. Two weeks later the volunteers returned to eat the other type of carrot, so that the urine samples could be compared. Researchers found out, that subjects who were fed the modified carrot absorbed up to 41% more calcium per 100 g carrots, than subjects who were fed normal carrots (Hawthorne, 2009).

	Milk	MOD carrot	CON carrot
Intake (mg calcium)	38	40	35
Serving volume	30 mL	65 g	120 g
Fractional absorption*	50.1 ± 3.0%	42.6 ± 2.8%	52.8 ± 3.3%
Total abs/100 g	64 mg	26 mg	15 mg
Relative servings**	240 mL	10 carrots (650 g)	15 carrots (1000 g)

All data are Mean ± SEM
MOD=biotechnologically modified; CON=control
*Milk vs MOD, $p < 0.05$, Milk vs CON, $p = 0.7$.
**This serving size of milk provides 300 mg of calcium.

Table 1: Absorption of calcium for milk, modified (MOD) carrots, and control (CON) carrots

Progress made

The background enabling this genetic manipulation lies in the discovery of the sCAX1 calcium antiporter from 1996. The group of scientists (Dr. PhD. Hirschi et al.) was able to create a carrot which provides higher calcium levels. The calcium provided by the carrots can be absorbed byns (Hawthrone, 2009). Dr. PhD. Hirschi mentioned in the interview that they succeeded in applying this technique to other vegetables such as potatoes, tomatoes and rice.

Relevance for preventing osteoporosis

As explained by Dr. PhD. Hirschi, this is only a very small step in the right direction. Certainly, people who ate these carrots and thus increase their calcium level would benefit from them. Still, the prevention of osteoporosis requires the public to get more calcium, vitamin D and more exercise. The amount of calcium stored in a carrot is not sufficient to provide enough calcium for people.

Mistrust in public

One noteworthy challenge when looking at genetically modified foods in general is, as mentioned before, the acceptance in society. There is still a big mistrust in our culture when it comes to genetically modified foods and possible problems they might create. This study was made in the years up to 2008 and made a big step forward in showing the effectiveness of genetically modified foods and the application of the used techniques. It is important to educate people on the applied techniques to build trust.

Future research steps

The research was stopped in 2008, due to mistrust in genetically modified foods as explained in the paragraph above. In the Interview Dr. PhD. Hirschi said that he would like to look further into where exactly the calcium is stored. As he also mentioned in the interview, they are still not completely sure as to whether the calcium is stored in the cell's vacuole.

Discussion of ethical aspects

Advantages

This kind of engineering technique is fairly easy and widely used. Also, many different genes can be induced into other plants through Agrobacteria. This could enable us to occupy plants with different properties, such as enhancing nutritional values or making them more resistant against pests. In times when climate change threatens us more and more, we could also adapt plants to rougher weathering conditions. The benefits of genetically modified plants are therefore numerous. This specific application of genetic modifications would contribute to osteoporosis prevention.

Limitations

According to Dr. PhD. Hirschi, genetic modification could enable carrots to absorb other toxic substances, if the soil they grow on has high levels of such substances. This means that it is not clear whether the sCAX1 antiporter could also exchange hydrogen ions with toxic substances. Also, as mentioned before, the amount of calcium stored in a carrot is not sufficient to provide enough calcium for people.

5. Summary

In the battle against osteoporosis a carrot has been produced to help people to absorb more calcium. Through gene transformation with the help of Agrobacterium tumefaciens, the sCAX1 gene, found in *Arabidopsis thaliana* was genetically modified and transferred to carrot cells. This gene codes for sCAX1 antiporters, which exchange hydrogen ions with calcium. The modified carrots show higher calcium levels than normal carrots and the calcium they provide can be absorbed by humans. The amount of calcium stored in a carrot is not sufficient to provide enough calcium for people. Therefor these carrots are only a very small step towards the right direction. Genetically modified foods are still highly controversial, and people do not wish to eat them. On the other side they are already being used in agricultural sectors such as in cotton production.
An interview was conducted with Dr. PhD. Kendall Hirschi, who worked on this study, in which he explained mechanisms and supplied information for composing this paper.

6. References

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Figure 2: Pittman, J.K, Hirschi, K.D. (2001). Regulation of CAX1, an Arabidopsis Ca^{2+}/H^+ Antiporter. Identification of an N-Terminal Autoinhibitory Domain. Plant Physiology, 127(3),1020-1029. URL: http://www.plantphysiol.org/content/127/3/1020

Figure 3, Figure 4, Figure 5, were created with: https://biorender.com

Table 1: Hawthorne, K.M., Morris, J., Hotze, T., Hirschi, K.D., Abrams, S.A., (2009). Biotechnologically-modified Carrots: Calcium Absorption Relative to Milk. J Bioequiv Availab, 1, 034-038. URL: https://www.longdom.org/open-access/biotechnologically-modified-carrots-calcium-absorption-relative-to-milk-jbb.1000006.pdf

Picture 0: Uncyclopedia contributors. Carrot. In Wikipedia, The Free Uncyclopedia. URL: https://en.uncyclopedia.co/wiki/Carrot [3.2.2021]

Picture 1: Wikipedia contributors. Arabidopsis thaliana. In Wikipedia, The Free Encyclopedia. URL: https://species.wikimedia.org/wiki/Arabidopsis_thaliana [3.2.2021]

Picture 2: Apsnet.org, Crown gall, URL: https://www.apsnet.org/edcenter/disandpath/prokaryote/pdlessons/Pages/CrownGall.aspx [.2021]

7. Attachments

Interview

1. **Why did you use carrots for your research?**
   Carrots can be eaten raw and we knew the transgene worked in this plant. Remember: cooking can affect Calcium levels
2. **How does the gene sCAX1, deriving from the model plant Arabidopsis encode calcium? Why did you choose this plant?**
   I published a paper in 1996 PNAS isolating the gene by its ability to work in yeast to move Ca into the vacuole. So I knew it worked.
3. **Had the sCAX1 gene been known before?**
   Yes see #2
4. **What technique did you use to induce carrots to express increased levels of sCAX1? How does this technique work?**
   Agrobacterium transformation. This bacterium infects the plant and leaves DNA in the plant. We engineered the DNA to encode sCAX1.
5. **How long did it take to create carrots which provide more calcium?**
   They need 2 years to regenerate, and make sure that they are stable.
6. **What difficulties did you encounter during the process of development of the carrot?**
   Carrots are tough to grow and the transgene stability is an issue. Some lines did not keep the foreign DNA.
7. **Where is the calcium stored, which the modified carrot supplies?**
   In vacuoles, we think, but we have not looked precisely.
8. **Did you try to apply this method to other vegetables?**
   Yes, we have made potatoes, tomatoes, rice etc. using this technology
9. **I found research papers online from 2008. Is the research being carried on and if so, at what stage are you currently? If not, why?**
   People are not interested in GMO foods, they are scared of them. We are not currently working on this project ☹
10. **What are future research steps?**
    I would like to look at where the Ca is in the plant – your question #7!
11. **Are there any known dangers with this type of genetic modification?**
    The danger if this is grown in soils with toxic metals is that the plants may accumulate more of those metals
12. **What are the advantages?**
    Advantage: more calcium in the foods
13. **Would you add these carrots to you diet?**
    I would eat these carrots.
14. **How effective is the enhancement of calcium intake when it comes to the prevention of osteoporosis? Do we already have any data base here?**
    Osteoporosis requires that the public get more exercise/vitamin D/ and eat way more Calcium. These carrots would represent a VERY VERY small step in the right direction
15. **What motivated you to work on this project?**
    I am interested in making healthier foods by using biotechnology