SUPER STRAWBERRIES

»We should eat strawberries with fish in them?!«
»No! «
»Then why did you say there is fish protein in these 'Super' Strawberries? «

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Preface

Who doesn't love strawberries?

Strawberry pie, strawberry jam, strawberries and cream… the list could go on.

We believe that strawberries are the best of the berries. The bright red-coloured fruit has always been associated with purity, passion, healing and of course it's magnificent taste. It has been used in literature, paintings and in an uncountable amount of dishes through the ages. Not only ancient Romans were familiar with their sweet aroma. The American Indians were already eating strawberries when the Colonists arrived. And still to this day, strawberries are amongst one of the most popular fruits.

The garden strawberry (Fragaria x ananassa) is a widely grown hybrid species of the genus Fragaria. A widely unknown fact however is, that the strawberry fruit is not, from a botanical point of view, a berry. Technically it is an aggregate accessory fruit belonging to the rose family.

Unfortunately, in colder countries like Switzerland these beloved fruits only grow in the warmer months of Mai to September. This means that domestically produced fruits are unavailable for the remaining half of the year.
But what if strawberries could be grown throughout the entire year? What if they could resist the cold temperatures of the winter?
The idea of frost-resistant strawberries has been floating around the department of gene-modification for a few years now. Is it even physically possible to create a a strawberry that can withstand and grow in cooler temperatures? With genetic engineering? - Maybe!
This is a task that scientists and researchers have been working on for years now and there have been a lot of trials and errors but a few successes as well.

This paper will be looking at scientific history, possible engineering techniques and ethical aspects as well as include an interview of a professor who specialises in the breeding and genetics of strawberries and blueberries.

Introduction

The strawberry is a major berry crop around the world. Since years researchers and scientists have tried to optimize the production of strawberries so that the berry could reach its maximum potential in aspects such as the yield, fruit size and quality traits, such as colour and taste.
“However, the narrow genetic base of the cultivated strawberry, combined with the polyploid[1] nature of the crop constrains traditional breeding methods.”[2]
With those obstacles in mind, scientists in biotechnological fields have taken the path that leads through genetic engineering. And the results prove this way to be an efficient alternative strategy to accomplish strawberry improvement.

One significant problem in the production of strawberries is the frost damage.
The producing of strawberries happens best in temperate climate. This however limits the time in which strawberries can be cropped and harvested.
Most strawberry crops begin in spring, however early spring frost damages the flowers leading to poor yields and erratic fruiting. Additionally, strawberries are soft and fragile products and furthermore they have a very short shelf life. To cope with these rather negative traits the strawberry fruit must be stored at low temperatures for an extended shelf life but at freezing temperature, ice crystals can significantly damage the strawberry.

Thus, scientists were looking for methods and techniques regarding the protection of strawberries against frost.
Prior to this research the properties of antifreeze proteins[3] have known to be efficient in the field of frost resistance, as the name already suggests. This protein seemed to be a potential candidate for the frost-resistant factor in strawberries.
Hitherto, the antifreeze protein of arctic fish was experimented with and its expression in the strawberry fruit turned out to be successful.

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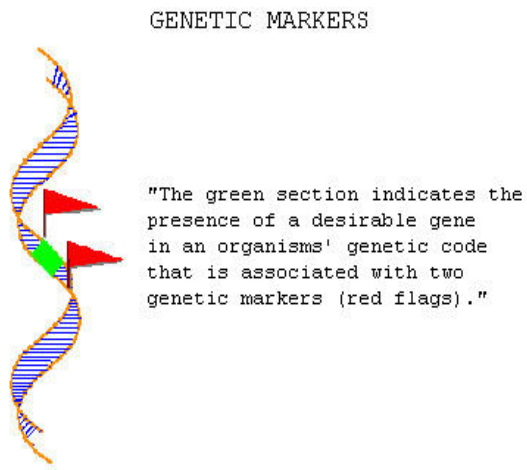
[1] containing more than two homologous sets of chromosomes

[2] http://rdo.psu.ac.th/sjstweb/journal/27-4/02-strawberry-gene.pdf (04/01/18)

[3] (or ice structuring proteins (ISPs)) refer to a class of polypeptides produced by certain vertebrates, plants, fungi and bacteria that permit their survival in subzero environments (https://en.wikipedia.org/wiki/Antifreeze_protein (04/01/18))

The engineering technique: transfer of the AFP into the Strawberry

As we now know, the transfer of genes encoding antifreeze proteins from Antarctic fish is one means of increasing frost resistance. But before we go into detail on how this exactly takes place, we'll briefly explain some background genetic information of the correlation of the strawberry and the antifreeze protein:



Applying approaches based on molecular markers[4], researchers can now more quickly identify the genes associated with frost tolerance. Such markers are used in molecular genetics to identify a specific sequence of DNA or proteins that show heritable variation.
The scientists examine several strawberry varieties under controlled cold-temperature studies in the laboratory to pinpoint the relevant markers. They have found a clear link between frost resistance and the amount of proteins in the plants. (*Fig. 1)*

In conclusion, a high amount of certain proteins encoded in the genes of the strawberries could lead to a successful frost resistance ability.
But rather than a transfer of the protein, the 'correctly edited' genetic material coding for the protein (the complementary DNA) is added to the receiving cell.

[4] In genetics, a molecular marker (identified as genetic marker) is a fragment of DNA that is associated with a certain location within the genome. Molecular markers are used in molecular biology and biotechnology to identify a particular sequence of DNA in a pool of unknown DNA. (https://en.wikipedia.org/wiki/Molecular_marker (04/19/18))

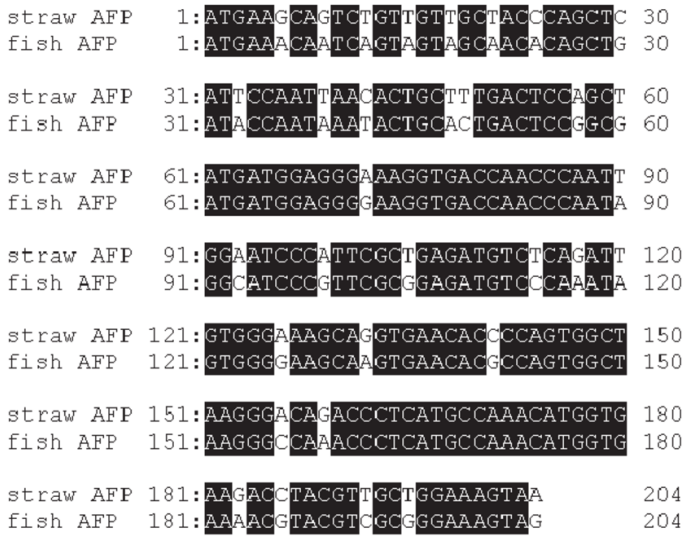


Fig. 2: A comparison of base sequences between strawberry optimized codon and original fish AFP cDNA. Bases identical between these two sequences are boxed.

However, high levels of heterolo - gous[5] protein expression in plants is not routinely achieved. One of the critical factors that limit the expression rate is codon usage[6], due to limited availability of particular tRNAs.

It has been necessary to optimize codon usage of several of the genes encoding for bacteria used as bioinsecticides, in order to achieve high expression level in plants. Therefore, it may be possible to increase expression of the antifreeze protein from fish (most commonly used is arctic flounder) in strawberry, by optimizing codon usage, to mimic highly expressed strawberry genes.

The purpose of modifying the antifreeze protein gene originally from fish was to create a modified gene that would be expressed strongly in strawberry cells. The sequence of the wild type antifreeze protein gene would be replaced with the strawberry bias codons, without changing the amino acid composition. The base sequence of this gene compared to original AFP gene from fish is shown in the figure on the left.

[5] In cell biology and protein biochemistry, heterologous expression means that a protein is experimentally put into a cell that does not normally make (i.e., express) that protein. (https://en.wikipedia.org/wiki/Heterologous (04/19/18))

[6] The frequency with which a particular organism uses the available codons in genes.

Documentation and pictures of the Department of Horticulture in the College of Agriculture and Natural Resources, *Michigan State University and interview with Jim Hancok*

Answers to questions of Elif and Nadine:

1. Please state a detailed description of your job as a scientist. (In what field do you work exactly? (For how long?)

I have a PhD in plant genetics from the University of California. I recently retired as a professor at Michigan State after 38 years and now manage a private blueberry breeding company – Berry Blue. I have taught courses in general genetics, breeding and genetics, and crop evolution. My research has revolved mostly around the breeding of blueberries and strawberries using both traditional and biotechnological approaches. A lot of my work has been involved in utilizing wild germplasm in crop improvement.

2. Being a specialist in breeding strawberries is quite an exciting and fascinating occupation. How did you come about this field of work? And why?

Believe it or not, I was actually studies to be an evolutionary biologist, but my major professor was a famous strawberry breeder Royce Bringhurst. I observed from him the wonder of plant breeding. When I took the job at MSU I was not hired to breed strawberries and blueberries, but over the years redirected my research efforts based on what I had learned from him.

3. What exactly does someone like you do on a daily basis? Do you currently work on projects or research projects?

While at MSU, I probably spent 50% of my time in various aspects of breeding research and writing, 25% teaching courses and advising graduate students, and 25% on other departmental and university service. During the growing season, I generally spent a full day each week visiting my breeding plots in the field. Now, I actually work from home interacting via the internet with our breeding scientists and visiting them once a month or so.

4. As our place of interest lays in the technique and use of genetic engineering of frost-resistant strawberries, we would like to ask some questions regarding this topic:

- **Why is the breeding of frost-resistant strawberries even such a big research? Would frost-resistant strawberries change aspects of farming and selling to a significant amount? Would it also influence other aspects?**

Almost every year in temperate climates at least some flower buds are killed by winter cold or spring frosts. Some years the damage can be particularly severe if snow cover in the winter is minimal or a freeze occurs while flowers are open in the spring. Damage can be mitigated by covering plants with straw over the winter or overhead irrigation during frost in spring.

- **Does the market sell these kinds of strawberries nowadays? If not, is it because there still needs to be research done or does the government not allow the selling of genetically modified strawberries?**

Strawberries grown today are much more winter hardy than those grown in the past. These improvements have come through conventional breeding. However, flower bud resistance to frost has been little improved.
There are no genetically modified strawberries now being sold, but it remains possible to get a GM strawberry through the government regulatory structure. It just has not been done to date.

- **Would you as a scientist approve or disapprove of the selling of frost-resistant strawberries? Why yes/Why no?**

Sure, if they were deemed “safe” by our government regulatory structure.

- **A technique that is known today for the breeding of the special strawberries is the transfer of an Arctic Fish Antifreeze Protein (AFP) into Strawberries to Increase Frost Resistance. How did scientists come up with this idea? Was the property of this gene being frost-resistant known prior to the research?**

I don’t know the scientists, so I really can’t comment on what drove them towards this idea. It does seem logical that this could be one way to improve strawberry frost resistance, but it would face a steep regulatory path.

- **What progress was made with the application of this technique? Are there other techniques regarding the breeding of frost-resistant strawberries?**

It never was deployed, as far as I know. It was done as a proof of concept and never was introduced anywhere. I am not even sure that they ever proved the incorporation of the gene did enhance frost tolerance. They just proved they could insert it into the strawberry genome.
Yes, there are other possibilities. We played around with a gene called CBF1 from Arabadopsis some years ago, but it was not all that effective. No attempts were made to commercialise the process.

- **What are the future research steps?**

I still don't think we have a likely candidate to improve the frost hardiness of strawberry, but I have been out of this area of research for 10 years or so.

- **Are there any major advantages or disadvantages of these strawberries or of this research (E.g. expensive/cheap, not enough/plenty of material, disagreement/ approval from the side of the consumer, etc.)**

Since we do not have a good source of resistance to frost in the native germplasm of strawberry, GM approaches could be helpful. Finding a conventional source of frost resistance has been problematic, so a GM approach holds promise. However, we need a quality candidate gene to move forward.

A photo of the building for plant biology of the Michigan State University.

A photo of one of the researchers preparing for experiments on the strawberries.

Discussion

As our interview partner already said, this technique hasn't been applied, in a commercial way at least (there are some lab reports that report the appliance of this technique). So, we don't know the consequences when they would be sold at the market. As there are different ways of experimenting on frost-resistance, the study with of the transfer of the antifreeze-protein hasn't had the chance to develop and make a significant progress.

As mentioned, there are no genetically modified strawberries on the market, since there is a strict government regulatory structure, also regarding GMO in food general.

And supposing that they've complied with all regulatory requirements, the consumer would still pose a problem, as many are still wary with the concept of GMO.

This fear of something yet unknown to them is only partially justified, as GMOs only have a potential risk of allergic reactions, when a new protein is introduced to the human diet. Because the protein that is being created within the Frost-resistant strawberries originate in Arctic Flounders, fish that are commonly consumed, there is an extremely low possibility of having an allergic reaction due to this crop.

This crop may also cause problems to consumer due to religious and/or moral beliefs because according to some beliefs, genetically modifying an organism of any kind is unnatural and immoral.

Surprisingly, there would be several advantages if these strawberries were to be sold.

Frost-resistant strawberries reduce the need to use chemical pesticides because a gene has already been inserted into the plant to prevent what the pesticides would fight off, such as harmful bacteria. Furthermore, the crop will help farmer's economical stand point because they do not have to waste money on purchasing and using pesticides to protect their strawberries.

These strawberries are more marketable to the consumer because the strawberry has a brighter and healthier red tone, is firmer, and can grow larger than the ordinary strawberries. The strawberries are also modified to taste and stay better for longer.

Additionally, frost-resistant strawberries are beneficial to the economy because the farmers who take advantage of this crop will gain more money due to the greater yield and the consumers have cheaper, more available strawberries to purchase, thus a win-win for both sides.

Summary

In this paper we have looked at several aspects including general information about strawberries and their genetics, a possibility as to how strawberries could potentially be made frost resistant, an interview with a specialist in the breeding and genetics of berries and different aspects involving economic and moral advantages and and disadvantages.

Overall the idea of genetically modifying strawberries to be more frost hardy is great. It would result in greater yields over a longer period of time, meaning growth in the economic sector, and also enable farmers to use less pesticides if genes protecting the strawberries against harmful bacteria, for example, were also included.

However finding ways to create this genetically modified strawberry and making it frost-resistant has proven to be a great challenge due to genetic complexity and limited availability of essential supplies, such as particular tRNAs for example. These factors result in a slowed down process of research and development but scientists are and will keep on trying. And methods such as the insertion of antifreeze proteins which we've presented in this paper have shown faint successes and might potentially and successfully be developed into a product that could be sold on the international market in a few years.

Until that time comes however, we will need to remain patient and it will probably require further years or research, lots of trial and error and the persistence of scientists.
Who knows, maybe seeing the strawberry as a rare pleasure only lasting for a limited timespan of the summer months is even something great and makes us appreciate the delightful sweetness of the fruit so much more.

Sources

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