Fusarium resistant wheat? - NFP 59

Fusarium head blight (FHB)

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Preface

I chose this topic because I wanted to do something with basic food sources like wheat and corn. The humanity has a strong dependency on wheat because of products like bread, pizza, pasta, cakes and pastries just to name a few of them. My interest lies in finding out more about the ways in which modern biotechnological methods can be used to make crops like wheat immune to fungal pathogens of the Fusarium genus like *Fusarium oxysporum* or *Fusarium graminearum*. We consider this to be an important issue in agriculture as the use of genetic engineering against FHB is already being employed on a global scale in big countries like the United States, China or Canada for example, which we found interesting to find out as we had only been aware of the use of GMO's on a national scale. Hence, we will also try to take the current political and agricultural situation in Switzerland into account. Furthermore, we want to investigate and document such interesting techniques like breeding, screening, genetic variation and mapping.

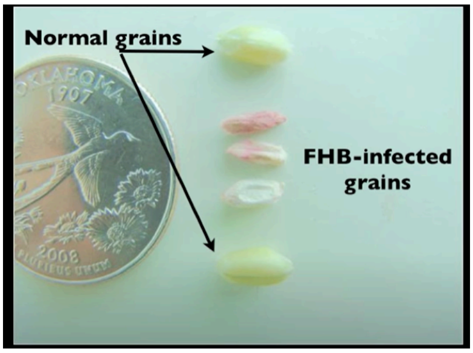
Introduction

What is Fusarium head blight, what makes it important and how does it affect wheat and other crops?

Fusarium head blight or FHB is one of the most common diseases in wheat and is very well documented and studied in science because it is well known for its devastating effects on wheat and other small grain agriculture. There are at least 17 different Fusarium species that lead to FHB, but it is mostly caused by the species *Fusarium graminearum*. Head blight affects the yields and quality of it while mycotoxins such deoxynivalenol and zearalenone are emitted, which contaminate the grain. It can be harmful to animals as well as humans. Certain environmental conditions can also favor the progress of the disease like high humidity, high precipitation and high temperatures at day and night. The spores are mostly spread by rain or wind. It is also important to know that the grain head will not instantly be infected, but only when it flowers. The spores can last on the residue of the plant for a long time where they will reproduce which they can do both sexually and asexually. It is at the flowering stage of the plant when it becomes vulnerable to the spores and ultimately becomes infected. The symptoms will start to show a week after infection. They include a bleached or white head as the figure below showcases.

Figure 1: Shows infected wheat head

The grains will also shrink and show the signature white or pink color as the following Figure demonstrates:



Fusarium and its mycotoxins can also infect Humans although they aren't likely to experience serious health problems from the pathogens. However, in most countries it seems to be important enough for there to be a law which implies a maximal percentage of mycotoxins in food, for example 100µg/kg = (1/10000) %. In USA, the Food And Drug Administration (FDA) recommends at most one part per million for a finished product for human consumption.

How is genetic engineering performed in Switzerland?

In Switzerland, the cultivation of GMO's[1] is widely prohibited to this day. The situation in the EU is similar where only import is partially allowed. In 2005, a standstill agreement (Moratorium) of 5 years was accepted through a popular petition which indicated that the growing of GMO's would be permitted for research purposes. The swiss federal council thereupon tasked the SNF [2] to start the research program NFP 59[3] to investigate the benefits and risks of genetically modified fusarium resistant wheat to swiss agriculture. Over the course of three years from 2008 to 2011, liberation field tests were performed. After the field tests were completed and data was fully analyzed, the swiss parliament decided, inspite of the results showing the standstill agreement twice by 5 years each in 2010 and 2015 and the swiss federal council has recommended for it to be extended once more to 2025.

However, after NFP 59 was officially concluded, liberation field tests were continuously performed since 2016, the "Protected Site" was founded by the Agroscope - a new site in Reckenholz with much improved security to prevent vandalism - where GM plants can be experimented with. Different genes and gene combinations and their interaction with the environment are analyzed. Currently six different projects have been conducted or are being worked on. Most of them have the aim to explore resistance genes of crop plants for specific diseases: The first research has been started in 2014 and is still being worked on and deals with resistance genes of GM spring wheat against mildew. In another project, research was done on cisgenic[4] potatoes carrying up to three resistance genes which have proven to be highly effective against potato blight (Phytophthora infestans) as the combination of multiple genes is very hard for the pathogen to penetrate. In a similar

[1] GMO = genetically manipulated organism
[2] GE: **S**chweizer **N**ationalfonds zur Förderung der wissenschaftlichen Forschung = Swiss National Fundings for the facilitation of scientific research
[3] Nationales Forschungsprogramm 59: "Nutzen und Risiken der Freisetzung genetisch veränderter Pflanzen"
[4] Cisgenic meaning that the genes were transferred from an organism that is related to the recipient

project, cisgenic apple trees with a resistance gene against fire blight[5] were being analyzed while a different field study explored, whether the yield of GM winter wheat could be increased with the help of a gene from barley. Two other projects both respectively are currently dealing with the resistance of maize and barley to fungal pathogens with help of a wheat gene.

Even though the current law permits these projects with GMO's, the staff has to file an application for every single project that they start which has to be authorized be the BAFU[6]. The process of writing an application and waiting for a decision usually takes over a year. This makes the beginning of new projects a very time-draining endeavor.

Alternatives to genetic engineering: Fungicides

In agriculture, the most used and simple method to neutralize fungal pathogens like those spread by Fusarium is the spraying of fungicides. However, as with most plant protecting agents, they can have severe impact on the health of the environment. Some of them take very long to dissipate, depending on their chemical composition and to a smaller degree on environmental conditions such as temperature, precipitation or light irradiation. They can last in the soil for a long period of time contaminating it and destabilizing the ecological equilibrium as they are mostly completely synthetic substances. The agents which are mobile when moving through the soil also pose the risk of eventually going into the ground water.

This is especially likely when there is great precipitation and a small or missing layer of plants covering the soil. In order to prevent this process from occurring, the environmental protection offices of each canton have defined protection zones in Switzerland where the use of these agents is forbidden.

Aside from the environmental hazards caused by Fungicides, they bring other disadvantages with them, such as the time frame of their application being very narrow and them therefore being quite inconsistent in their effectiveness. Also, resistant fungal strains can be developed.

It appears as if there is need for a better solution to fusarium pathogens in wheat. In 2018, a big step forward was made when the entire genome of wheat was sequenced. It was found to consist of over 15 billion base pairs and be separated into 21 chromosomes.

Description of engineering technique

As genetic engineering against FHB in wheat is currently not being implemented in swiss agriculture and the projects conducted by the Agroscope are dealing with different problems, we will write about its application in other countries. In 2005, a study published by The American Phytopathological Society (APS)[7] explained how the activation of the transgenic Arabidopsis thaliana NPR1 gene or AtNPR1 gene could effectively confer a heritable, type 2 resistance of wheat to the FHB caused by Fusarium graminearum. Type 2 resistance means that the disease symptoms spread less at the site of infection after it had received a point inoculation[8]. The AtNPR1 gene is derived from the chinese cultivar

5 Erwinia amylovora
6 **B**undes**a**mt **f**ür **U**mwelt = Federal Office for environmental protection
7 https://apsjournals.apsnet.org/doi/pdf/10.1094/MPMI-19-0123
8 Inoculation means, that a portion of the pathogen is being brought into the host

Sumai 3, which has a type 2 resistance against FHB, as the study explains in its introduction. In Arabidopsis thaliana or thale cress, which is also a host for F. graminearum, the overexpression of the NPR1 gene was observed to reduce growth of pathogens in its leaves. Hence, the hypothesis was formed that the NPR1 gene would increase defense mechanisms and it was chosen to be transferred. They continue to say that their general approach was to target regulatory genes which control the expression of one or more defense genes. By this method, they want to reach the state of a so-called System Acquired Resistance (SAR). SAR can be described as the activation of different pathogenesis-related (PR) genes and for it to develop, salicylic acid (SA) or benzothiadiazole (BTH) has to be applied inside the cell. The expression of the AtNPR1 gene in wheat was found to make the plant respond quicker to the SA without having any impact on its yield.

Wheat ended up showing a phenomenon similar to SAR, which was that after SA was applied to the plant and it had received an inoculation of Fusarium graminearum the PR genes would be activated and with that the resistance to FHB. The cultivar Bobwhite was chosen as the recipient of AtNPR1. The bar gene which gives the wheat resistance to glufosinate was used as a benchmark to identify the transgenic T_0 plants as stable, meaning that there is a low number of mutations in them.

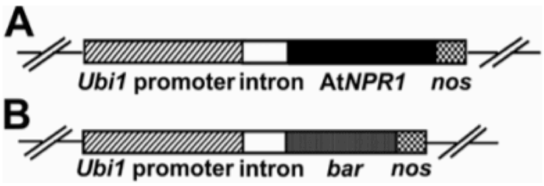


Figure 2: Shows the genes with promoter

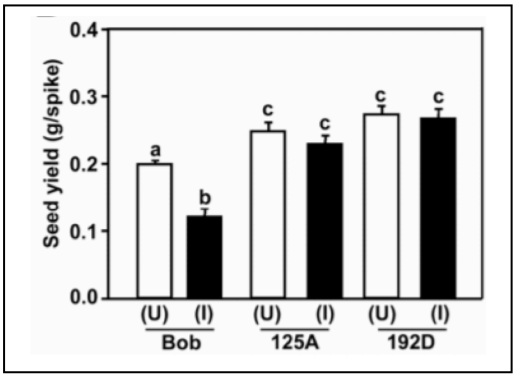
For both these genes, Ubiquitin1 of maize was used as a promoter. There were three such stable plants. In generations following these three plants, T_1 to T_4, the chimeric[9] genes were all stably inherited. However, in the offspring of one of the three, the AtNPR1 gene was not expressed leading to the speculation that it was silenced in one of the generations. For the T_4 plants, polymerase chain reaction was used to reaffirm, that AtNPR1 was present. They were then exposed to F. graminearum spores. The original Bobwhite cultivar and Sumai-3 were used as reference. As a result, the two plants with stable AtNPR1 expression showed a strong type 2 resistance to FHB. Afterwards, the T_4 plants were crossed with the Bobwhite cultivar. Their offspring all possessed the same type 2 resistance to FHB leading to the strong assumption that trait of resistance is dominant as the following Figure shows:

Plant	No. of plants tested	R	S
Bobwhite	12	0	12
125A	12	12	0
192D	15	15	0
F_1 (Bobwhite × 125A)	12	12	0
F_1 (Bobwhite × 192D)	14	14	0

9 Chimeric meaning that there is more than one distinct genotype to be found, in this case the one composed of AtNPR1 and bar

*Figure 3: **R** = **R**esistant to FHB, **S** = **S**usceptible to FHB*

Figure 4: 125A and 192D are the two transgenic plants with AtNPR1 expression,



***U** = **U**ninoculated **I** = **I**noculated (with F.graminearum)*

Also, the plants that didn't show AtNPR1 expression had no resistance to FHB. The second graph also represents the effectivity of this technique as the grain yield of the different species is shown[10]. In the end it was concluded that the reason why the wheat showed resistance to FHB was that the defense-related PR-genes were activated more quicker and stronger. This is due to the plant being more responsive to BTH.

Documentation and pictures of research

For the interview, we decided to ask questions about the situation in Switzerland, especially the political, and therefore it seemed reasonable to contact someone working in a department of education or research. We also wanted to make the questions rather generic. At first, we wrote Professor Wilhelm Gruissem[11] as we considered him a suitable interview partner. However, he referred us to his colleague Beat Keller[12]who in turn gave us the contact of his employee Teresa Koller with whom we then conducted the interview.

Interview with Teresa Koller[13]

What do you think about the current situation in the swiss agriculture?

In context with NFP 59 the conclusion was drawn, that the benefits of gene technology outweigh the potential harms. Unfortunately, in swiss politics this has been and still often is completely disregarded. From the standpoint of a researcher, it seems illogical to me that society and politics often either completely reject- or show great disinterest towards genetic crop engineering, as the sole purpose of it is to breed a new, better plant that, in this case, is resistant against (Fusarium) pathogens. In Switzerland Genetic engineering is generally forbidden to perform although researching it is happily allowed. Researchers are having a hard time understanding this because they want to contribute to better crops with less toxic chemicals.

[10]

[11]Wilhelm Gruissem: Biology department of ETH, specialized in plant biotechnology

[12] University of Zürich: Head of the Department of Plant and Microbial biology

13 Teresa Koller, University of Zürich: Department of Plant and Microbial Biology

What are the different approaches to immunity in wheat seeds?

The most common method is the use of fungicides to purge the seeds of fungal pathogens. But unfortunately, with crossbreeding, the process takes a very long time and therefore it is easier to perform genetic engineering. Also, one can only implement the genes we really need which is much harder with crossbreeding. However, the constant spraying of pesticides takes its toll as it can also harm important parts of the environment. The other thing would be maintaining a sort of rotating pallet of different crops. In the first-year wheat, second year corn, third year sunflowers and fourth year maybe potatoes. This ensures that every year there is a cut instead on sustaining and developing fungus in the ground.

Of the different methods, which ones do you consider to be the most sustainable?

At first, I would say the breeding is the most important because the plant itself can get a good resistance. So, my best solution would be how to teach the plants to defend themselves against the fungus. However, I think that genetic engineering could help to improve crop plants. It is very sustainable because it generates a better quality of those crops while reducing fungicides and pesticides to a minimum furthermore reducing the impact on humans. It is sometimes a bit sad that such great results from our scientists get “ignored” so to speak and nothing changes in the laws. I think we must open our possibilities as a country and start to use genetically engineered crop plants but while maintaining safety and scientific accurate guidelines.

What is your opinion on genetic engineering with crop plants?

In my opinion it is very good. There have to be laws that’s very clear because with every technique you could do bad things, but not as strict as it is right now. Also, it is beneficial because we are able to use less fungicide and therefore create potentially healthier food for human and animals while being very efficient and using less area per unit of food. I think it could be extremely useful rather than harmful but only in a controlled environment and with the right laws. Unfortunately, Switzerland has some of the strictest rules when it comes to genetically modified food and it seems unlikely that this will change soon.

Discussion

How much progress was made / can be made with this method?

The method of transferring the AtNPR1 gene can be applied to many different crop plants but it has also proven to be effective on grapes, carrots, tomatoes, apples, citrus fruits, tobacco, strawberries and rice and likely many more in order to protect them from fungal, viral or bacterial pathogens. Yet, in many cases research still must be done in order to identify the side effects that come with the employment of this technique.

What are the next steps?

As already mentioned, there is not enough research completed at this point concerning negative effects on the environment, animals and humans for certain transgenic plants to be introduced into large-scale agriculture. Conducting such research and eventually finding positive results could be very impactful when it comes to clearing up the concerns that are frequently addressed in political context. Especially in Switzerland and the EU where there is great opposition against the use of GMO’s it is crucial to provoke a paradigm shift in society.

Chances

Introducing transgenic wheat with the AtNPR1 gene to potentially worldwide agriculture could increase profits massively as the yield size of an infected transgenic plant was shown to be double that of an infected non-transgenic plant. Furthermore, the use of fungicides would also be replaced, to a certain degree which increases the health of the environment.

Dangers

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Summary

Fusarium head blight in wheat is a disease that is of great economic importance for agriculture and as the writing of this paper has made clear to me, finding a sustainable, long-term solution to it is of great interest for agriculture. One method is the transfer of the AtNPR1 gene found in *Arabidopsis thaliana.* However, it seems unrealistic to be employed in Switzerland in the near future as the cultivation of such GMO's is still illegal and also violently rejected by politics and society.

Personal Reflection

When looking through the paper, I get the impression that I perhaps emphasized the introduction (and the political situation) a bit too much and thus the part about the engineering technique became a bit sparse. I am also aware that the part about the discussion is incomplete.

It was fascinating for me to learn about the transfer of the AtNPR1 gene to other organisms and the unexplored possibilities that it holds. I also found it exciting to hear about the political situation in Switzerland and how it could continue to unfold.

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