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Projekt titel:

HealthWheat

Development of a low allergenic, high nutritional organic wheat

Table of content

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Problem and idea behind the project

Gluten intolerance, obesity and other health problems related to the consumption of wheat are increasing in an almost epidemic way in modern societies, and many consumers chooses organically grown heritage varieties to minimize the risk linked with modern wheat production. However, heritage varieties are not necessarily better suited for organic conditions or having a better health profile. As demonstrated in our own previous research and now also confirmed in the ongoing project "Klokt Mot Sot" (Borgen *et al.* 2018), resistance to common bunt and other diseases of special relevance to organic farming, are rarely found in Scandinavian heritage varieties. Additionally, it was found in the HealtGrain project, that varieties with elevated content of beneficial dietary fibre was found in varieties and landraces of exotic origin rather than in our own heritage varieties (Shewry *et al.* 2014).

The grand vision of this project is to combine several different beneficial traits from both heritage, exotic and modern varieties to develop low allergenic and healthy wheat cultivars adapted to organic farming in Scandinavia. The vision is to decrease the number and severity of negative reactions experienced by a large proportion of the consumers when eating wheat and improve wheat production in organic farming. As this project focus on combining several different traits, it differs from most projects that study only one or a few traits separately.

Hypothesis

We believe that the increase in food related allergies and intolerances to wheat and gluten in recent decades, is not explained by a simple cause. Rather, it is a combination of multiple factors that has gradually changed over the years. The factor that have changed over the decades are thus both internal and external changes such as lifestyle, food processing techniques and pesticide use. Plant breeding of modern wheat varieties is part of the problem but also can be part of the solution, if the above issues are considered in the plant breeding process.

In different regions of the world, existing and new genotypes of wheat with decreased content of different allergenic compounds have been identified or developed within the last 5 to 10 years. In addition, wheat genotypes with increased content of dietary fibres and different antioxidants have been identified. Our hypothesis is that by crossing genotypes with desired traits in an organic breeding program, these beneficial traits can be combined into genotypes adapted and suited to organic farming in Scandinavia to benefit both organic farmers and consumers.

Goal

Overall goal is to test if the increase of food allergies can be reduced by development of low allergenic and healthy wheat cultivars adapted to organic farming in Scandinavia. As this extends the timeframe and budget of the grant, the project goal is to develop 5000 organic pre-breeding lines combining low allergenic and high nutritional properties. These pre-breeding lines will constitute the basis for further work needed to reach the overall goal.

How the project is relevant with respect to the aims of the foundation

The project will increase human health by supporting the development of better food. Food that supports the body to perform a healthy and natural digestion and absorption of food. By developing healthier wheat varieties with less allergens and more dietary fibre and beneficial metabolites such as antioxidants, we hope to halt the increasing frequency of people experiencing negative effects on their health and wellbeing after intake of wheat, and to limit the negative reactions in people currently suffering from wheat related allergies and digestion problems.

To also minimize the negative effects of pesticide use, we envision the organic sector to be frontrunners in the development of new wheat varieties with less allergens and better nutritional profiles. We need organic plant breeding to be visionary and tackle the challenge of delivering this plant material. We believe, that this work is necessary for the continued development and expansion of organic lifestyle and food. The plants developed in this project is targeted for the organic production system.

The health problems associated with consumption of wheat is increasing, and the mechanisms behind the problems are understood better than before. Preventing the occurrence of these problems require a holistic view on human health, including lifestyle, food processing and food production. This project will support this view on human health.

Present situation, current international knowledge/research and conclusions

Wheat has been one of humanities most important sources of food since the neolithic stone age. Wheat contains both important proteins and energy, and because of the high yield, wheat can feed many people on a limited farming area.

In our modern world, people are increasingly experiencing problems caused by consumption of wheat. Modern wheat genotypes and processing techniques cause severe health problems for a substantial part of the population. Kucek *et al.* (2015) estimated that 11 – 38 % of people is suffering from fructose malabsorption, and 11,5 – 14,1 % is suffering from irritable bowel syndrome (IBS). Lowell and Ford (2017) found that IBS was found to in 1,1-45,0% of people to a higher or lower degree with varying occurrence between countries. IBS is, beside fructans, caused by Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols (FODMAP) (Staudacher and Whelan, 2018) and can therefore be caused not only by wheat.

On top of these numbers, it is estimated that 0 – 5,6 % of all people are suffering from celiac disease, with regional differences in prevalence. In Sweden between 0,5 – 2,9 % of the population is suffering from celiac disease (Leonard *et al.* 2017). However, there is also a portion of the population who does not have celiac disease, but who still cannot tolerate wheat. Some of these are suffering from nonceliac wheat sensitivity (NCWS), a condition where the innate immune response seems to be triggered, but where the cause and mechanism still not fully understood (Leonard *et al.* 2017). The actual frequency of nonceliac wheat sensitivity is unknown because the current lack of biomarkers makes the condition difficult to diagnose, but it is thought to be more common than celiac disease (Leonard *et al.* 2017). As a complicating factor, people suffering from IBS are at increased risk of having or developing gluten or wheat sensitivity. Therefore it is difficult to distinguish IBS and gluten sensitivity (Catassi *et al.* 2017). Additionally, 0,2 – 0,5 % of the population suffers from wheat allergy such as Baker´s asthma and atopic dermatitis (Kucek *et al.* 2015).

Because of the many human health problems associated with wheat, we need to take a critical view on wheat as a food source. Wheat is characterized by a relative high content of starch and a low content of dietary fiber, resulting in a high glycemic index causing problems with obesity, diabetes and cardiovascular diseases (Bell and Sears 2003). Most of the proteins in wheat are in the gluten fraction, the storage proteins for wheat. The gluten fraction is also the proteins responsible for the superior baking quality of wheat. The gluten fraction consists of gliadins and glutenins. Glutenins are divided into two groups namely the Low Molecular Weight (LMW) glutenins and the High Molecular Weight (HMW) glutenins. The HMW glutenins are among the largest naturally occurring proteins. Gliadins are smaller molecules than glutenins and is divided into α-, γ- and ω-gliadins. Both glutenins and gliadins are difficult to fully digest by intestinal enzymes. Specific digestion-resistant gluten peptides have been found to cause an immune reaction in predisposed individuals (Kucek *et al.* 2015). The α- and ω-gliadins are the most common reactive components in wheat, shown to be involved in celiac disease and wheat allergy. However, γ-gliadins and LMW as well as HMW glutenins are also reported as reactive in some cases of celiac disease and wheat allergy (Kucek *et al.* 2015). Another group of proteins in wheat have also been shown to be a major allergen: The α-Amylase Trypsin Inhibitors (ATIs) (Kucek *et al.* 2015, Pastorello *et al.* 2007). ATIs are involved in both celiac disease, wheat allergy, and nonceliac wheat sensitivity. ATIs are part of the plants defence against pathogens and pests (Wand *et al.* 2011). Earlier, the assumption was, that ATI was denatured by the heat during the baking process, but recent studies have shown that many consumers respond to ATIs in bread and other grain-containing foods (Junker *et al.* 2012, Heiden 2018B).

The problems with these wheat proteins are manifold: Firstly, the mentioned proteins are resistant to digestive enzymes. So, without adequate number of enzymes and time prior to and during digestion, they will pass though the digestion system partly undigested and thus be in their reactive form when they come in contact with the intestinal mucosa. Secondly, in modern bread techniques, the fermentation period is very short or absent partly due to addition of synthetic enzymes, and often dominated by only one microorganism, namely baker´s yeast (*Saccharomyces cerevisiae*). The lack of a diverse microbial digestion, lack of fermentation time and lack of the associated development of acidity, as is occurring with traditional sourdough fermentation, greatly reduces the pre-digestion hydrolysis of reactive proteins (Kuzek *et al.* 2015). A traditional sourdough fermentation is also more efficient in reducing the content of the problematic fructans (Kuzak *et al.,* 2015). It is further hypothesised that a cocktail effects of pesticide residues and mycotoxins contribute to the increasing intolerance to wheat and to other food intolerance syndromes. Changes in the mycotoxins are potentially caused by pesticide mediated change in mycoflora in the crop canopy. Organic farming relies on plant resistance to diseases and does therefore not contribute to these negative effects.

Fibers in the food increases the time the food spend in the digestive tracts and thus increase the time the digestion enzymes have to degrade these difficult-to-digest proteins. Water soluble fibers such as β-glucan, pentosanes and arabinoxylans are known to be especially healthy in modern diets, and recognized to decrease diabetes, obesity and cancer. However, the amount of soluble fiber in wheat is lower than in other Scandinavian grains such as barley, oat and rye, but there are differences among varieties. The Chinese variety Yumai 34 and a few Turkish landraces has been identified with superior content of soluble fibers (Shewry *et al.* 2013). Breeding based on these lines has been initiated by Agrologica since 2015.

In recent years, several research groups around the world have screened accessions of wheat for their α- and ω-gliadin content and for type and quantity of ATIs combined with their immune-reactivity. The screenings have also included fructans contents and substantial differences have been found in all characters (Kuzak *et al.* 2015). For example, the Keyserlink Institute in Salem, Germeny has identified Goldritter and other varieties with a low content of ATI based on tests performed by Labor von Prof. Schuppan in Mainz, Germany (Heiden 2018A, Heiden 2018B). A screening of 324 varieties from various parts of the world performed by Nakamura *et al.* (2005) identified the variety ´CM32859´from Mexico as the least allergenic variety. Both lines will be included in the breeding program at Agrologica from 2018.

Wheat contain several antioxidants, including vitamins, phenolic compound, anthocyanins and carotenoids. Carotenoids is found in the endosperm giving the wheat flour a yellow appearing known from example durum wheat and einkorn, but it can also be found in bread wheat. Carotenoids are pre-stages of vitamin-A, and via gene manipulation, Syngenta and others agrochemical companies are promoting the GMO “Golden Rice” in Asia and Africa to improve human health. However, elevated content of β-carotene and luteins are already found in the natural occurring genepools of wheat. Agrologica identified some of these lines and have already developed breeding lines of bread wheat with elevated carotenoid content from natural gene sources.

Flavenoids and especially anthocyanins are strong antioxidants known in several fruits like blueberry, elderberries, red grapes and many others, but are also found in special types of wheat known as purple wheat and blue wheat (Beta *et al*. 2011). Specific properties make anthocyanins attractive in prophylaxis and therapy due to their anti-tumor, cardio- and hepatoprotective, antimutagenic, antiulcer, UV-protective, and other health beneficial effects (Kong *et al.* 2003, Kay 2006, Prior and Wu 2006, Hodgson and Croft 2006). It appears that these phytochemicals are responsible for the reduced risk of various diseases associated with oxidative stress, such as cancer and cardiovascular and neurodegenerative diseases (Jacobs and Steffen 2003). Agrologica started breeding for increased content of anthocyanins already in 2008 and have advanced breeding lines of both purple wheat and blue wheat.

However, we want to cover a wider range of low allergenic properties and increased nutritional components. We therefore apply for this project. The least allergenic lines found in the screenings will be collected for the project and included in a breeding program designed to combine the desired traits in genotypes suited to organic farming conditions in Scandinavia.

We acknowledge that plant breeding alone cannot secure healthy food, and we therefore want to promote the new healthy wheat lines together with organic farming, whole grain products and natural fermentation techniques.

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CV of Project participants

Agrologica is a small research and breeding company based in Mariager, Denmark that has specialised in organic farming, plant genetic resources and plant breeding. The company was founded by PhD Anders Borgen and include technical staff and PhD Nanna Ytting, who is in charge of quality assessment of the cereal program.

Anders Borgen has been a certified organic farmer since 1984 and is well known in the organic community in Scandinavia, and with a PhD on common bunt in organic wheat production and a long list of successful on-going and terminated projects on seed research, plant pathology and plant genetic resourced, he has a strong base for running the breeding company for organic farming. Anders Borgen has published 125 post graduate publications mainly on seed health, organic farming and plant genetic resources, and has been involved in 40 externally funded research and innovation projects, including 15 projects as scientific and financial project manager. A full list of publications and projects can be found at www.agrologica.dk.

Anders Borgen has been involved in European networks on seed saving and heritage wheat varieties since 1998, including the Let’s Liberate Diversity Network, and cooperated with Hans Larsson and Allkorn since 2002 on reintroducing heritage varieties to the marked and organic production both as a farmer and as a researcher. Together with Hans Larsson and others, he has organised the annual events Nordic Heritage Cereal Seminar since 2008.

In 2006, Agrologica changed from mainly selecting within heritage cereal varieties, and started a targeted breeding program of wheat and other cereals for organic farming. We now have a portfolio of advanced breeding lines selected for organic conditions based mainly on agronomic performance and baking quality. We wish now to include also the human health aspect described in this application.

Material and methods

Based on existing literature, a search for low-allergenic wheat lines and lines with better nutritional profile will be conducted and the lines will be acquired. The company Agrologica already have CM32859 (low allergenic), Goldritter (low ATIs) and Yumai34 (high fiber) and a wide range of breeding lines with elevated levels of anthocyanins and lutein.

The acquired lines will be crossed during summer 2019 (traditional breeding methods) to combine the desired traits. To speed up the breeding process, the first generation (F1) will be grown in Chile where the summer period occurs from September to March. Agrological have an established arrangement with the Chilean company Semillas Generación 2000 Ltda., who specializes in this type of work. We will personally visit the Chilean farm to ensure that this step proceeds according to European organic regulations.

In summer 2020 the F2 generation will be grown and selected for field performance in organic plots in Mariager, Denmark by Agrologica. When material have reached F4 and beyond it will be uniform enough to be screened for desired health traits. When it reaches F8 it begins to be uniform enough to do large scale multiplication and final validation of quality. However, this work lays beyond the current project period and budget.

Plant breeding is a long process taking many years and thus the work will extend beyond the current project period. Agrologica have already started the breeding process of combining some positive traits to create healthy wheat. This work includes combining traits such as dietary fibers, anthocyanins and lutein. This project therefore aims to do the further steps to combine these traits with more traits such as low ATI, low αand ω-gliadins and low fructans. The work therefore consists of doing the crosses between parental material and to grow, select and multiply the offspring.

We believe that the entire production chain, from plant breeding to final product, need to be considered when producing healthy food for the body and soul. We aim to combine promotion of the new healthy wheat lines with promotion of organic farming, local food production and natural fermentation techniques.

Expected results and how the project will be documented and disseminated

The expected result is 5000 organic breeding lines in F3 which have parental material with different combinations of low-allergenic and high nutritional profiles. These breeding lines will be the foundation for the development of low allergenic, high nutritional wheat adapted to organic farming in Scandinavia. At this early breeding stage (F3), it is not feasible to do analysis of allergens and health promoting substances. This selection will be done on more advanced breeding lines.

The project will be promoted at relevant cereal conferences and meetings such as Nordisk Kornseminar, The European organic wheat gathering “Let’s Cultivate Diversity 2019” a.o., and at the company homepage. The lines will be shown at the yearly open demonstration of Agrologica´s field trials. Promotion and documentation will also be used to attract cooperation partners for the further breeding work. Another long-term goal is also to attract corporation partners that can study and document the combined health benefits of the new wheat line with optimal fermentation and processing techniques.

Time plan, budget and information about financing from other organizations.

To develop a new genotype takes app. 10 years/generations of breeding and testing. We apply for support only to the first steps in this pre-breeding work; namely make the initial crossing of wheat genotypes and then grow and select the offspring according to performance in the field.

The project is in its initial phase and currently not financed by other organizations.

Expense type	Cost (SEK)
Salary 2019*	161.200
Salary 2020*	156.000
Other expenditure, including soil rent, field trials, propagation in Chile during winter, equipment maintenance	60.000
Travel and accommodation, including local travel and visits to the Chilean farm	50.000
Total	***427.200***

Total project budget, *Hourly salary: 520 SEK

Month	**Jan 2019**	**Feb 2019**	**Mar 2019**	**Apr 2019**	**May 2019**	**Jun 2019**	**Jul 2019**	**Aug 2019**	**Sep 2019**	**Oct 2019**	**Nov 2019**	**Dec 2019**	***Total hours per work task***
Literature search	30												*30*
Acquire genotypes		20											*20*
Seed genotypes				40									*40*
Manage plots with genotypes					30	30							*60*
Cross genotypes							50						*50*
Harvest and thresh								50					*50*
Visit Chilean farm						30				30			*60*
Total hours per month	*30*	*20*		*40*	*30*	*60*	*50*	*50*		*30*			***310***

Time budget for year 2019. Numbers indicate hours.

Month	**Jan 2020**	**Feb 2020**	**Mar 2020**	**Apr 2020**	**May 2020**	**Jun 2020**	**Jul 2020**	**Aug 2020**	**Sep 2020**	**Oct 2020**	**Nov 2020**	**Dec 2020**	***Total hours per work task***
Seed F2				40									*40*
Manage plots with F2					60	60							*120*
Evaluate and select best plants, F2					20	20	20						*60*
Demonstration of field trials							20						*20*
Harvest and thresh F3								60					*60*
Total hours per month				*40*	*80*	*80*	*40*	*60*					***300***

Time budget for year 2020. Numbers indicate hours.