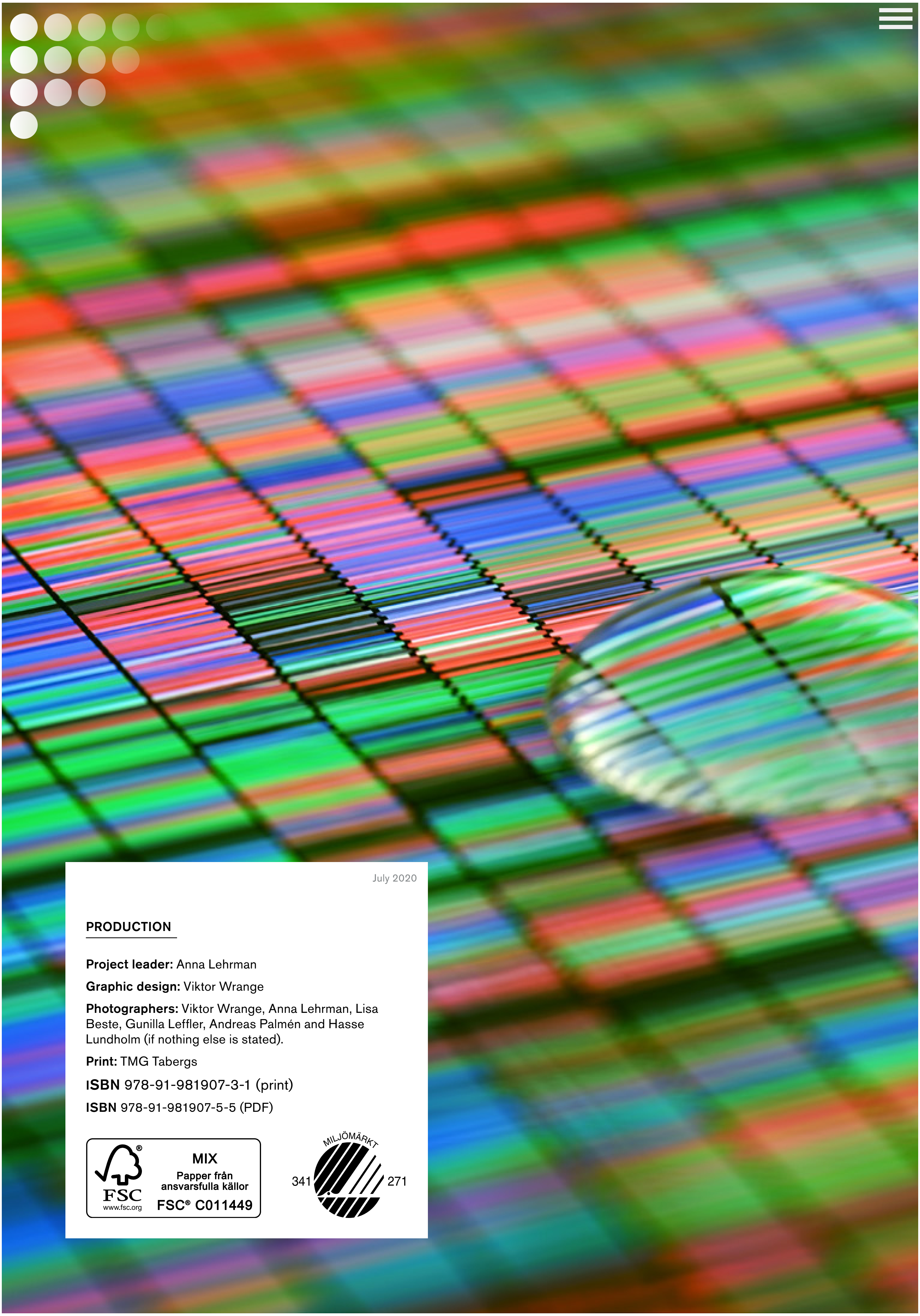
SLU
Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Biotechnology for Sustainability

Final Report from Mistra Biotech 2012-2020

MISTRA Biotech



July 2020

PRODUCTION

Project leader: Anna Lehrman

Graphic design: Viktor Wrange

Photographers: Viktor Wrange, Anna Lehrman, Lisa Beste, Gunilla Leffler, Andreas Palmén and Hasse Lundholm (if nothing else is stated).

Print: TMG Tabergs

ISBN 978-91-981907-3-1 (print)

ISBN 978-91-981907-5-5 (PDF)

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MAIN FUNDERS

MISTRA
The Swedish Foundation for
Strategic Environmental Research

SLU

The world faces major challenges associated with our environment, human use of natural resources and our impact on our surroundings. The Swedish Foundation for Strategic Environmental Research (Mistra) plays an active part in meeting these challenges by investing in the kind of research that helps to bring about sustainable development of society. This is done by investing in various initiatives in which researchers and users make joint contributions to solving key environmental problems. Mistra's programmes cut across disciplinary boundaries, and the results are intended to find practical applications in companies, public agencies and non-governmental organizations. For more information, vistit www.mistra.org.

What is Mistra Biotech?

In June 2009 the Board of Directors of The Swedish foundation for strategic environmental research (Mistra) decided to invite proposals for a programme about biotechnology for the environment, including the social and ethical aspects of applied biotechnology. Further discussions resulted in a call that had its focus on agriculture and food systems. A proposal from SLU, in consortium with Lund University and the Royal Institute of Technology (KTH) won the competition, and in 2012 the research programme Mistra Biotech was launched with SLU as the host. Over the eight years more than 100 researchers have been involved in Mistra Biotech, either through a funded position or through some other form of collaborations. Most of us have been affiliated at SLU, but some of us work at KTH, Lund University, Roskilde University, Uppsala University, the University of Copenhagen or the University of Gothenburg. 2020 marks the end of Mistra Biotech, and the time has come to summarize our research.

Our main focus has been on the use of biotechnology for agriculture and food systems that are sustainable from ecological, ethical, social, and economic perspectives. Mistra Biotech has been highly interdisciplinary and unique in the way it has provided a platform for direct and close interactions throughout the programme period between researches in the natural sciences, social sciences and humanities.

Our research in the social sciences and humanities have dealt with a wide range of issues involved in the introduction of biotechnological products in the food system. Our studies of the ethical and legal issues that are involved have been performed in broad co-operations involving ethicists and legal scholars as well as plant and livestock breeders. We have explored the implications of the precautionary principles and the ethical aspects of concepts such as naturalness and technology neutrality. We have investigated the potential reception of biotechnological products among farmers, the food industry and consumers. We have also performed comprehensive studies of the potential introduction of new crop and of the potential use of biotechnology to reduce disease resistance in livestock and thereby improve animal welfare.

Our research in the natural sciences has been aimed at utilizing the potential of agricultural biotechnology to contribute to a more sustainable food production with healthier food products, reduced environmental impact, and less disease-ridden farm animals. Three of the largest projects in the programme have been (1) breeding for late blight resistance in potato, (2) breeding for a more healthy potato with altered starch composition, and (3) the domestication of field cress (*Lepidium campestre*) as a new oil and cover crop with several improved agronomic traits. In this research, genetic improvement of target traits has been obtained with conventional breeding, genomic selection, traditional genetic modification and gene editing (site-directed mutagenesis) including the CRISPR/Cas9 technology.

The animal breeders in the programme have improved the use of molecular information in livestock breeding (genomic and proteomic selection). Co-operation between animal and plant breeders has been essential in transferring methods in genomic selection from animal breeding, for which they were originally developed, to plant breeding. Both plant and animal breeders have been in close contact with the ethicists and social scientists in the programme, which has led to mutual influence on the research performed.

The research in Mistra Biotech has been well integrated in SLU's research and teaching. It is closely connected with other on-going research, and much of it will continue in other projects and programmes.

“We need to satisfy the environmental targets, succeed with climate adaptation, and at least not increase our dependency on food imports.”

– Inger Andersson

Words from the Chair

"Mistra invites Swedish research organisations and companies to submit proposals for a research programme concerning sustainable development of agricultural and food systems using biotechnology as a tool. Biotechnology is defined broadly. The overall purpose is to promote a more sustainable society within a timeframe of 20–40 years."

These were the first words in the call for applications in April 2011 that led up to Mistra Biotech. Surely, some wondered what biotechnology could have to do with sustainable development. But with each year that has passed it has become more and more clear that this was an insightful and far-sighted initiative.

It has been fascinating to follow the researchers' work during these eight years. Modern technology provides us with new ways to make our food more healthy and its production more sustainable. With small and well-directed mutations, potato can become resistant against late blight, which leads to a large reduction in the need for pesticides. The same method can provide us with potatoes that are high in fibre, and therefore more healthy. Without biotechnology this would not have been possible.

In livestock breeding, the DNA of young stock is analysed at an early stage in order to determine which of them are suitable for breeding. In this way, breeders can obtain results such as improved animal health at a higher pace.

We have also followed the domestication of a new crop. Field cress, previously only known as a wild plant with small white flowers, is gradually transformed into a biennial oil crop, adapted to withstand the climate also in the northernmost parts of the country.

All of this is important research. We need to satisfy the environmental targets, succeed with climate adaptation, and at least not increase our dependency on food imports. Plant and animal breeding have crucial roles in achieving this. Fortunately, we have all the competences in these areas that we need.

But unfortunately there is a hurdle. We have a European legislation on GMO that in practice makes it impossible to use the results of this research. A court judgment in July 2018 made it clear that the strict rules for transferring genes between plants also applies to directed mutation, for instance with the new technology CRISPR/Cas9. This is not easy to understand, since this technology can produce exactly the same type of mutations that take place in nature all the time. It is not possible to determine afterwards if a mutation was obtained with biotechnology or if it originated in the random processes of nature. The end result will be the same.

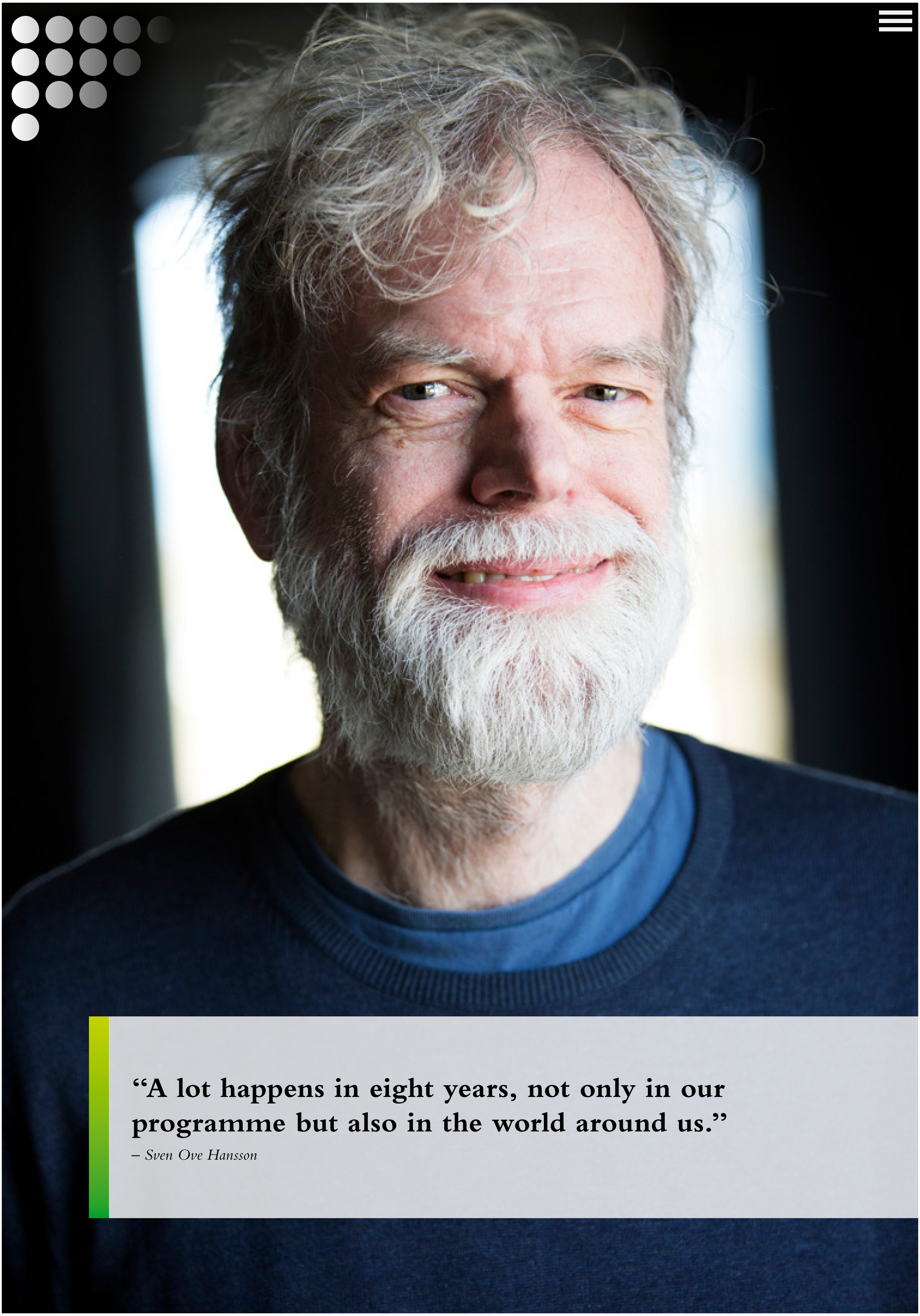
This is not a matter of environmental protection or precaution. What we have is an out-dated legislation that puts bureaucratic obstacles in the way of much needed research and innovation. We urgently need a modern legislation that makes it possible, for instance, to grow new potato varieties with much reduced need for pesticides.

But the researchers in Mistra Biotech have not despaired. Instead they have worked constructively with alternative proposals for a revised legislation that strongly protects health and the environment, without obstructing innovations that contribute to sustainability. They have clarified the application of the precautionary principle, developed ethical principles for plant breeding, analysed the media debate, and investigated how consumers perceive information about the technology used to produce their food.

We are often told that researchers have great difficulties in cooperating across disciplines. Our experience is different. The work with legislation and ethics has largely been performed in working groups where geneticists, plant breeders, and animal breeders have cooperated with ethicists, legal scholars and social scientists. New thoughts emerge more easily if different ways of thinking meet and – if I may say so in this context – cross-fertilize.

The long list of publications speaks for itself. We know from all the presentations on the board meetings that we have first-rate researchers who are strongly committed to continue working for a more sustainable agriculture in Sweden. If the bureaucratic barriers can be removed, then chances are good that we will achieve what Mistra asked for in their call for research applications in 2011.

Inger Andersson
Chair of the Board, Former Director General
of The Swedish National Food Agency



> “A lot happens in eight years, not only in our programme but also in the world around us.”
>
> – *Sven Ove Hansson*

WORDS FROM THE PROGRAMME DIRECTOR

Where do we go from here?

I am writing this in the 99th and last month of Mistra Biotech. We have worked together for more than eight years, and I think we can be proud of what we have achieved. We have reported significant results from our laboratories and field trials, as well as from our desk-based research. We have built exciting co-operations between disciplines that seldom co-operate. We have communicated with decision-makers and the public, and we have been listened to. And we have had great fun.

A lot happens in eight years, not only in our programme but also in the world around us. I would like to point to three important developments that contribute to reshaping our research field.

GENE EDITING

The first of these is gene editing. When our programme started, the first gene editing tools such as TALEN and zinc finger nucleases were just emerging. Now, gene editing in the shape of CRISPR/Cas9 is rapidly reshaping biological and medical research as well as practical applications – plant breeding as well as clinical medicine. With gene editing, it is possible to mimic nature's own major way of modifying the genome, namely changes in a single base pair, the smallest unit of a gene. The big difference is that in nature, such mutations occur randomly, but with gene editing, we can specify exactly where this change will take place. For instance, if a specific gene is responsible for a protein in a plant that a pest needs to damage the plant, then that gene can be blocked with editing tools such as CRISPR. This is much faster and more reliable than traditional methods to achieve pest resistance.

Importantly, there is no way to determine if a single mutation in a gene has been obtained with gene editing or if it is the result of a (random) natural mutation. This is no problem for safety, since the risk that the mutation brings some undesired trait is the same, independently of how the mutation was obtained. But this also means that the current European legislation, which raises virtually insurmountable formal obstacles to the use of gene editing in plant breeding, is increasingly out of touch with reality.

CLIMATE CHANGE

The second of these developments is climate change. Agriculture is more dependant on the climate than any other major branch of economic activity. Unfortunately, the situation has worsened considerably during the programme period. When the programme started, we saw a growing awareness of the urgency of the problem, and in 2015 the Paris agreement gave us some hope of a development in the right direction. But two years later, a new, blatantly science-denying, American administration struck a heavy blow at all these efforts by announcing its withdrawal from the agreement. We are now in a situation when every lost year in climate change mitigation makes even more far-reaching measures necessary in the future. With every year, more and more far-reaching adaptation measures will be required in addition to the increasingly urgent and belated mitigation measures that we have to take.

This has immediate consequences for both plant and livestock breeders. A sustainable agricultural production in a changing climate will put high demands on breeders. They will have to develop crops with traits suited to the new climatic conditions, not least resistance to pests and diseases that will thrive in a new climate. It is unfortunate that governments around the world have defunded public breeding activities, which are now needed more than ever. Hopefully, the recent upscaling of publicly funded plant breeding in Sweden through SLU Grogrund is the beginning of a new trend that will facilitate the climate adaptation of Swedish agriculture.

Another lesson must be learned from the failures of international climate change policies. Science denial plays a crucial role in the politics that block urgent mitigation measures. After the hard learned lessons from climate policies, we cannot afford to let science denial take command in other areas. This concerns our own research area. There is a well-grounded and well-established scientific consensus that the effects of a change in the genetic code depends on what the new genome looks like, not on the means by which it was altered. Policies have to be based on science-based risk assessments that take this into account. Failure to do so can deprive us of means that we need to produce the food that the world needs when agriculture is threatened by climate change and new pests.

THE VIRUS

The third development is of course the covid-19 disease. When writing this, we are at the beginning of what threatens to become a much larger pandemic. This has immediate consequences for us as researchers; it has delayed our work and made it necessary to cancel important meetings, including the final Mistra Biotech conference where we had planned to sum up our achievements and make plans for future co-operations. But the pandemic is sure to have much more far-reaching consequences on our societies, many of which we cannot now foresee.

There is one conclusion, however, that we can already draw from this. The covid-19 epidemic has taught us how vulnerable our societies are, and how dependent we, as biological beings, are on the natural environment that we live in. Hopefully, that will lead to strengthened measures to deal not only with epidemics but also with other threats to our societies. The production, importation, and distribution of food and feed is one of the critical sectors of society that has to be included in such deliberations. If we had to be reminded of this, the fact that groceries along with pharmacies have been the only shops to be kept open all over the world, in spite of far-reaching lockdowns during the pandemic, should be reminder enough. Much more attention must now be paid to potential threats to food production.

> **“There is no shortcut around engaging with the public in a respectful way, listening to their worries and concerns and finding ways to assuage them.”**

HOW DO WE PROCEED?

Given what we have learned from these events and from our ninety-nine months of research, what do we do now? First of all, we should not see the end of Mistra Biotech as the end of a closed road, but rather as a junction with several roads that can bring us forward in the desired direction. The situation seems hopeful in terms of funding. There is a growing understanding in our society that plant and livestock breeding are indispensable. I am sure that we can qualify for research funding to continue the lines of research that we have developed in the past eight years.

On a global scale, agriculture faces huge challenges. Climate change will require substantial adaptations. Crops with increased tolerance to droughts and other stresses will be needed. Means must be found to protect livestock against new diseases that spread in a new climate. At the same time, increased demands for food have to be met, and this must be done without infringing on the already too small areas of wilderness that are still left on the planet. This means that productivity, in particular yield per hectare, will have to be increased. However, we certainly do not want to experience once again the negative environmental effects that have accompanied some yield increases in the past. All this adds up to what is indeed a difficult equation. One thing is sure: we cannot solve it without much intensified breeding activities. Governments need to take responsibility for ensuring sufficient resources for this as well as other activities needed to ensure a sustainable food production for the future.

In addition to all this, plant breeding has a public relations problem. People are worried that scientists create plants that endanger the environment, result in less healthy food products, or make subsistence farmers more dependent on large multinational companies. These worries have to be taken seriously. There is no shortcut around engaging with the public in a respectful way, listening to their worries and concerns and finding ways to assuage them. Increasingly, plant breeders can deliver crops with substantial advantages for health and the environment. There is no reason whatsoever to give up on convincing the public of the usefulness of these new crops and varieties.

So let us intensify our efforts to secure the resources needed to take decisive further steps towards a sustainable future agriculture. Let’s step up our explorations of new and exciting ideas on how it can be achieved. Let’s develop new co-operations, and pursue the social and ethical issues that need to be solved. And let’s enhance our dialogues with the public. Mistra Biotech was a good beginning.

Sven Ove Hansson
Programme Director, Professor in Philosophy
at the KTH Royal Institute of Technology

Our main achievements

Perhaps our most important achievement is the broad interdisciplinary approach that we have developed. Plant and livestock breeders have developed unusually tight methodological collaborations. Ethicists, legal scholars, media and consumer researchers have co-operated closely with plant and animal breeders in concrete research projects, leading to joint publications. All this bodes well for future research on agricultural sustainability.

We have studied the attitudes of consumers, farmers, and other actors in the food chain. We found that attitudes to food biotechnology are more nuanced than what is commonly believed. We have also penetrated the major ethical issues involved in agricultural biotechnology. This has resulted in new analyses of issues such as naturalness and precaution, and to the beginnings of a new ethical approach to plant breeding that puts focus on traits and breeding goals. We have performed detailed analyses of the increasingly outdated European legislation on agricultural biotechnology, and proposed what we see as realistic ways to improve and modernize it.

We have presented a strategy for how plant and livestock breeding can contribute to public health. This is an example how we have combined different areas of knowledge. The strategy puts focus on foodstuff with properties that satisfy the three criteria that breeders can provide them, that nutrition scientists recommend them, and that consumers will expectedly choose them.

We have developed new methods for animal breeding, using genomic information more efficiently to breed for animal health and other desirable traits. In a novel collaboration, we pioneered the application of proteomics to male reproductive success in cattle and identified for the first time peptides that could be future biomarkers for bull fertility. We have also laid the foundations for a new breeding strategy that makes use of cow data instead of relying exclusively on the breeding values of bulls. We have also investigated the pros and cons of using gene editing to make animals resistant against common diseases.

We have taken large steps towards the domestication of field cress as a new oil and cover crop, suitable for the colder Nordic climate. Our breeding goals have included oil content, different oil qualities, seed yield, perenniality, pod shattering, and seed germination. We have made major achievements with all the major breeding goals, and we have also combined several desirable traits into single breeding lines. Field trials have shown promising results. While unusually fast progress in domestication of field cress has been made during these eight years, further improvements of the domesticated species are needed before it can be introduced in agriculture as a viable crop.

We have bred potato with healthier (slower and resistant) carbohydrates, and we have also a proof of concept of a developed potato starch with improved properties as a raw material for bioplastics. We have investigated the genome to understand what affects the susceptibility of potato to late blight, a pathogen that currently causes much of the pesticide use in Swedish agriculture. To solve this problem, we have developed new potato lines that are promising with respect to disease resistance and the reduction of pesticide use. We have also studied new ways to increase nitrogen uptake in potato, which is important to avoid eutrophication.

Our work on both field cress and potato has required detailed investigations of the genomics and proteomics of these species, as well as the development and application of new breeding technologies. We have also performed studies on both oat and barley, mainly as a contribution to breeding for resistance against major pests and diseases that affect these species.

Our research results are presented in the publications listed on pages 104-113. We have communicated extensively with decision-makers, stakeholders, NGOs and the society at large, through publications, program website, social media, podcasts, and numerous meetings, as well as visits to our field trials.

THE FOOD CHAIN: PRODUCERS AND CONSUMERS

- A study of decisions in the food chain on the use of biotechnology, showing that the sensitivity of anti-GM campaigns tends to increase as one moves down in the food supply chain from production to retailing.
- A study of farmers' attitudes to GM fodder, showing that their opinions are largely based on more general considerations than cost-savings and profits.
- A study of market power in the soy feed industry, showing that when conventional soy price becomes competitive in South America, livestock farmers stand to lose due to feed industry market power, but more so where GM soy imports are not allowed.
- A questionnaire-based study showing that Danish farmers are more positive than Swedish farmers to GM crops. Swedish farmers are more concerned

about the impact of genetic engineering on nature, while Danish farmers are more distrustful of multinational companies.
- A published meta-analysis showing that European consumers are (on average) not more negative to food biotechnology than consumers in other parts of the world. Previous claims to the contrary seem to be the result of different types of questionnaire questions being asked in different regions.
- A study showing that that consumers tend to be more influenced by the uncertain risk factors than they by potential benefits of biotechnology in food products.
- A study showing that Swedish consumers are more likely to accept GM food if it is produced in the country and it has mandatory labelling.
- An experimental study of consumers' choice, of food items, showing that (1) they do not in general notice the label on packages of food products, (2) they pay more attention to negative than to positive properties of the products, (3) are influenced by persuasive information on GMO if it matches the individual's initial risk perception, and (4) are influenced by information on choices made by other actors in the food chain, especially retailers.
- A study showing that publications on GMO in food and agriculture in Swedish media have broadened their inclusion of topics over time, and gradually narrowed their geographical focus from a European to a Swedish perspective. Issues concerning agriculture in the Global South have not received much coverage.

ETHICS

- An ethical analysis showing serious shortcomings in the so-called hubris argument, according to which genetic modification of other species is always an inappropriate thing for human beings to do.
- An ethical analysis of the notion of naturalness, emphasizing the multidimensionality of the concept and proposing ways to indicate degrees of naturalness in food labelling.
- An article on how the precautionary principle has been applied to GMOs, arguing that an update based on the currently available scientific information is urgently needed.
- A tentative version of a mid-level precautionary principle: 'Reasonable precautionary measures should be taken to safeguard against uncertain but non-negligible threats'.
- An article proposing a framework for a practically oriented ethics of plant-breeding. Its main focus is on plant traits, breeding goals, and the rights of breeders and farmers.
- A proposal for how to evaluate ethical tools and to choose among the available tools for ethical analysis of biotechnology.
- A study of the ethical aspects of genetic modification and genome editing in livestock breeding, arguing for careful evaluation before such methods are introduced, and suggesting that animal breeding organizations should set up ethical committees.

PUBLIC HEALTH AND THE ENVIRONMENT

- We present a strategy for plant and animal breeding for public health. Some of the main recommendations are: (1) Target both micronutrient deficiency and diseases such as overweight and coronary disease. (2) Give the highest priority healthy variants of traditional food items that can be introduced universally. (3) Create efficient incentives to produce healthier foodstuffs. (4) Use the best available technologies. (5) Combine breeding with economic development, treatment of diseases that aggravate malnutrition, and adequate dietary information. (6) Reform the food production chain so that consumers' demands for healthier products have a stronger impact.
- A study showing that most identified effects of GM crops on ecosystems are indirect effects of traits that could also be carried by non-GM crops.
- A report showing that there are no significant environmental gains from importing non-GM soy over GM soy. However, if imported GM soy substitutes locally grown EU protein feed then negative environmental impact is expected.
- An analysis showing that the problems with coexistence between organic farming and farming with GM-crops, are linked to the labelling requirements. We suggest that effects on biodiversity should be a major consideration in the construction of labelling requirements.

LEGISLATION AND REGULATION

- An article showing that the current European legal framework for GMOs serves as a barrier to the market, in particular for less resource-strong actors such as small- and medium-sized companies and plant breeders working at universities.

We propose changes in the EU policy on GM crops that could help loosen the political stalemate that slows down the development of sustainable crops.

- An article providing a framework for determining whether or not a certain genetic alteration can be said to occur naturally.
- An article showing that the current EU policies and regulations for site-directed mutagenesis differ radically from those in other countries, in ways that may have considerable implications for international trade.
- A study of the situation for gene edited plants in the European Union, given the CJEU judgment in the case C-528/16. The lack of an event specific detection method creates considerable problems for the implementation and enforcement of specific regulations for such plants.
- An article focusing on rapeseed and rapeseed derived products. It indicates that the current GMO legislation may constitute an obstacle to innovative activity concerning these products.
- An article showing that the specific needs of Nordic agriculture cannot be expected to be catered for by the private sector. Therefore, public investments in crop breeding for this region are necessary in order achieve a sustainable agriculture.
- An article on the origin of the European GMO legislation, showing that important considerations that were taken into account in the original documents have now been lost: a focus on traits, periodic updating of the legislation, acknowledging a history of safe use, and acknowledging benefits from GM technology.
- A series of three articles analysing in detail various elements in the European legislation that can realistically be modified in order to make the legislation more predictable and innovation-friendly.
- An article analysing the effects for plant breeding of a Dutch proposal, according to which plants that have been developed with genetic engineering should nevertheless not be regulated as GMOs if they do not in the end contain any new genetic material.
- Two articles proposing an opt-in mechanism that would allow countries wishing to cultivate GM crops the opportunity to do so, provided that the crops in question have first been assessed as being as safe as a conventional counterpart by the European Food Safety Authority (EFSA).
- An article proposing a definition of technology neutrality, which is a central concept in many discussions on agricultural biotechnology.
- An article analysing the European GMO legislation from a legal point of view. We found shortcomings in the legal certainty and clarity of the legislation, and proposed ways in which it can be improved.
- An article reporting a comparison between four realistic scenarios for introduction of new cultivars in Europe, showing that the legislation is inconsistent from the perspectives of risk assessment and risk management.

ADVANCES IN ANIMAL BREEDING

- Full partnership in the 1000 bull genomes consortium, bringing global exchange of genetic information.
- A method for incorporating prior biological knowledge in genomic selection models by estimating different weights for SNP effects based on their biological function.
- A novel selection strategy that employs genomic data in crossbred sows to improve maternal and welfare traits. We have created a simulation program based on real genomic data.
- A new breeding strategy where the breeding values of the bulls are based cow data instead of the breeding values from 'old bulls'.
- A simulation study of what benefits genomic prediction, in combination with sexed semen and crossbreeding can deliver at the farm level.
- Identification of a mutation that results in a missing bit (important for animal health and fertility) of chromosome 23 in individuals of the Swedish Red White Cattle (SRB) dairy breed.
- Identification of 1000 proteins in seminal plasma samples, 38 proteins of which differ significantly in abundance between bulls with low and high fertility, of which 18 proteins correlates with fertility indexes.
- Identification of genes affecting the reaction in uterine cells to seminal plasma, which could explain differences in fertility. Preliminary results indicate large differences between cows in the reaction to seminal plasma from different bulls.
- A simulation study of the introduction of genome edited disease resistant pigs into breeding programs: Resistance can be efficiently obtained, but inbreeding will increase drastically unless many genome edited animals are used.
- Development of molecular tools for genomic selection in both plants and animals.

DOMESTICATING A POTENTIAL OIL AND COVER CROP

- Breeding lines of field cress with an increase from 11 to 84 per cent of the healthy oleic acid in the oil of the seeds, a reduction of the unhealthy erucic acid from 20 to 0.1 per cent, and a decrease of linolenic acid from 40 to 2.6 per cent.
- Increased oil content in field cress seeds from less than 20 per cent to up to 27 per cent, obtained through selfing and crossbreeding.
- Increased the oil content in field cress seeds up to 30 per cent, obtained with genetic modification.
- Field cress lines that produce wax esters in their seeds, obtained with genes from jojoba.

We identified a mutation that results in a missing bit (important for animal health and fertility) of chromosome 23 in SRB cattle.

- Successful adaptation of the CRISPR/Cas9 method in field cress.
- Identification and blocking of glucosinolate transporters genes in field cress.
- Increased seed yield in field cress by 100 per cent.
- Field cress lines that mature three weeks earlier than the late types, making them suitable for northern conditions.
- Reduced pod shattering in field cress (*Lepidium campestre*), obtained with interspecific hybridization with *L. heterophyllum*.
- Reduced pod shattering in field cress, obtained through genetic modification.
- *L. campestre* × *L. heterophyllum* interspecific hybrids that can be harvested several times with adequate seed yield.
- Identification of field cress genotypes with root-system that are favourable for breeding for perenniality.
- Identification of genes in field cress that regulate vernalization, florescence, pod shattering, and quantity and quality of the oil.
- Identification of two major fungal pathogens in field cress: dark spot (*Alternaria brassicicola*) and gray mold (*Botrytis cinerea*), and of breeding lines with resistance against these pathogens.
- Analyses showing that field cress is rich in phytosterols, which are known to reduce blood cholesterol levels.

- Hybrid field cress lines with stacked traits. They combine high seed yield, pod shatter resistance, synchronous maturity, and increased oil content.
- Field cress lines that combine pod shatter resistance with excellent seed germination.
- Results from trials showing that undersowing is a preferable approach for field cress, improving its capability to compete with weeds.
- Results showing that growing pigs can eat feed with up to 12 per cent seed cake from field cress, mixed with a common cereal-based feed.
- Development of efficient protocols for genetic modification and gene editing in field cress.
- Identified quantitative trait loci (QTLs) for stem growth orientation, plant height, number of stems per plant and perenniality.
- Partial sequences of 30 genes coding for major traits in field cress.
- Determination of the chemical composition of field cress seeds.
- The discovery that when field cress seeds are soaked in water, they form a gel containing pure pectin, a substance used in the food industry.

A HEALTHY AND SUSTAINABLE POTATO

- A strategy for table potato breeding in the Fennoscandinavian region.
- Potatoes with more amylose and with amylopectin that has a modified structure making it more similar to amylose. This results in healthier, slow or even resistant, carbohydrates.
- Adaptation of the CRISPR/Cas9 method of genome editing to potato.
- Potato lines with CRISPR-Cas9-induced mutations in all eight alleles, leading to a high amylose-only starch content.
- Analysis of GM lines showing that an intermediate amylose content increased the resistant starch content several fold.
- A study showing that the resistant starch content in the modified potato was influenced both by the amylose/amylopectin ratio and by the amylopectin structure.
- Showed that the change in starch content increased nitrogen uptake.
- Proof of concept that the modified amylose potato can be used for production on bioplastics.
- Development of proteomics tools and their application to potato (resistance to *Phytophthora infestans*, causing late blight).
- Robust DNA markers for pathogen resistance in potato.
- A number of potato lines based on King Edward, the dominating cultivar used in Sweden, obtained both through gene editing and traditional GM.
- A methodology for effector screening in practical potato breeding material.
- Elucidation of the key resistance mechanism for the potato line SW93-1015.
- Identification of proteins that seem to be of high importance for *P. infestans* at different life stages.
- A system for investigating immunity responses in potato.
- A method that reduces the number of SNP markers needed for genomic selection in potato, thus reducing the costs while still covering variation in any breeding population Identification of protein markers in potato for traits for which there are no commercial DNA markers yet (yield and resistance against *P. infestans*).
- Establishment of the genome editing technique TALEN in potato.
- A standard protocol for creating new potato lines and producing seed tubers for field trials within one year.
- Identification of an amino acid transporter that may increase nitrogen efficiency in several species.
- Demonstration that amino acids in soil are used as nitrogen sources by plants.
- Results showing that salicylic acid is necessary to restrict *Alternaria* growth and early blight symptom development in both potato tubers and foliage.
- A system for quantification of blackleg symptoms on shoots of *in vitro* potato plants.

CEREAL CROPS FOR THE NORDIC CLIMATE

- A model of the effects on climate and the environment if a barley with higher nitrogen use efficiency were introduced in Sweden.
- Identification of quantitative trait loci (QTLs) that are correlated with the level of *Fusarium* mycotoxin in infected oat.
- A full simulation of an oat breeding program showing connections between genotypes and the derived phenotypes, based on defined QTLs in the genome.

THE INTERDISCIPLINARY EXPERIENCE

Thoughts of a philosopher and a geneticist

Elisabeth Jonas, or Lisa as she is usually called, came to Sweden as postdoc the day before the first Mistra Biotech meeting, and she has been engaged in the programme since then. Now she is an associate professor in quantitative genetics with a focus on animal breeding, and during the years in Mistra Biotech she has been collaborating with plant scientists, economists, social scientists and philosophers.

– I appreciate the interdisciplinary nature of Mistra Biotech. It has been fruitful for the research itself and also interesting for me. I have learned to really listen to others and also to talk at a level that most people can understand, she says.

As an example she mentions the advantages of following the development of new technologies together with colleagues in an interdisciplinary network. During the Mistra Biotech years, the gene editing tool CRISPR/Cas9 has revolutionized the breeding research area.

– In the beginning, the potential application and development of gene editing in plant breeding was discussed a lot, but not in animal breeding. Now, when gene editing is the tool everyone is talking about also in animal genetics, I am already familiar with it, through the attention we have payed to gene editing at our programme meetings, says Lisa.

Another breeding method is genomic selection, which initially was developed with a focus on livestock breeding, before it was used on plants. In recent years the application of genomic selection in breeding of commercial crops has increased.

– I could contribute with my knowledge about how we select the best individuals within animal breeding, in a project where we use the genomic selection method in oats.

Associate Professor Per Sandin's research area is within applied ethics. Just like Lisa, he has participated in the programme from the start. He points out more advantages of being part of an interdisciplinary research programme, in addition to the purely scientific ones.

– Writing scientific papers together is one thing, but we have also learned from each other when the programme has arranged public activities, like the panel talks we have had at the Almedalen Week in Visby. At occasions like that, researchers from the different projects in Mistra Biotech have talked about the same theme but from different points of view, he says.

Per studies the ethical aspects of biotechnology. In Mistra Biotech he has collaborated with natural and social scientists as well as theological ethicists.

– Applied ethics is itself divided into disciplines. I have a background in philosophy. Collaborating with ethicists with a background in theology is in a way an interdisciplinary meeting too. We are working on similar problems with similar intellectual tools. At the same time, we have read very different parts of the literature and see problems in slightly different ways.

Lisa and Per agree on the importance of interdisciplinary meeting platforms for researchers to get a wider view on the scientific challenges. Especially when it comes to complex themes like biotechnology in agriculture.

– Biotechnology is a concern for many different sectors of our society. The development, use and consumption of a crop or a farm animal will have different kinds of consequences, ranging from biological to economical and ethical ones, says Per.

The programme has been running for eight years, and during this time the participants have had time to get to know each other's ways of thinking and looking at the world.

Before coming to Mistra Biotech, Lisa didn't have much experience in interdisciplinary work.

– Among other things I have gotten more insight how philosophers write scientific articles. It's so different from writing about animal breeding. Writing an article within natural sciences is very predetermined in terms of format, she says.

Per agrees with Lisa.

– Writing articles in natural sciences is almost like filling out a form. The writing is not the main part of the work. In philosophy and social sciences, on the other hand, writing is a major part of the research process. This is important to understand when we collaborate interdisciplinarily, he says.

Per Sandin, associate professor in philosophy, and Elisabeth Jonas,associate professor in quantitative genetics, highlight the importance of interdisciplinary meeting platforms.

Our interdisciplinary approach

We have put much focus on developing the interdisciplinary co-operations needed to solve our research tasks. The outcome of these efforts can be seen in a substantial number of publications co-authored by researchers from disciplines that seldom co-operate in that way. Our strategy for interdisciplinary research can be summarized as follows:

1. *The participants.* The scientists who wrote the application saw the need for co-operations between disciplines. Natural scientists in the programme were keenly aware that practical applications of agricultural biotechnology depend to a large extent on social and ethical factors. The social scientists and ethicists in the programme all wanted to base their own research on the most up-to-date natural science.

2. *Taking time to understand each other's methodology.* Beginning at the very first programme meeting, we have spent much time learning about each other's methodologies. The ethicists and social scientists have been introduced to the basic methodologies in plant and animal breeding and to modern biotechnology. Similarly, the natural scientists have been briefed on the methodologies used in applied ethics, consumer research and other social science disciplines. We have done this because experience shows that incomprehension of each other's methodology is a common hurdle in interdisciplinary research.

3. *Fostering a co-operative culture with high demands on scientific quality.* We do not see interdisciplinary studies as an "area in itself" in which some people have expertise. Instead, we conceive interdisciplinarity as co-operation between experts in different disciplines. This approach is essential to insure high quality. We also believe that participants' willingness to contribute to interdisciplinary work is strengthened when they see that their expertise is needed and that the contributions from other disciplines have high scientific quality.

4. *Forming interdisciplinary working groups to solve problems that require input from several disciplines.* We have identified research tasks that can only be solved with co-operation between disciplines, and we have formed working groups to solve those tasks.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Research

A summary of our major research achievments.

Biotechnology and the society

Mistra Biotech has put much focus on the social aspects of agricultural biotechnology. We have investigated the attitudes and perspectives of food producers and consumers, and we have studied the reporting and debate on biotechnology in mass media. We have scrutinized the major ethical arguments that have been put forward, and we hope to have added new important aspects to the ethical discussion. We have reviewed the potential effects of biotechnology on human health, and looked into a couple of examples of possible environmental effects. We have studied the pertinent European policy and decision-making practices, closely examined the legislation, and proposed several alternative ways to reform the legislation and the decision-making procedures for agricultural biotechnology.

Food producers

The actors in the whole food sector, *e.g.* farmers' organizations, individual farmers, retailers as well as the food processing industry all play important roles in forming a sustainable food production. The food producers' perceptions are formed within the frames of policies, legislations, wider public (including consumer) attitudes and demand. At the same time the food producers have the power to influence consumers.

VIEWS AND PERCEPTIONS AMONG KEY ACTORS IN THE SWEDISH FOOD SUPPLY CHAIN

How do key actors in the Swedish food supply chain define and operationalize the concept of agricultural sustainability? Who/what influences these organizations' sustainability policies and their respective positions on agricultural biotechnology? What are the organizations' views and perceptions of biotechnology and its possible role in creating agricultural sustainability? Based on policy documents and semi-structured interviews with representatives of five organizations active in producing, processing, and retailing food in Sweden, we investigated how these key actors perceive the concept of agricultural sustainability and the role of biotechnology in creating more sustainable agricultural production systems. We showed that external actors have a considerable influence on the policy process and the resulting conceptualization of the sustainability concept. In Sweden, the current dominant discourse says that biotechnology is not part of sustainable agriculture, at least not when it comes to food for human consumption. This is clear from the policies of the organizations participating in this study. A majority of the interviewed organizations claim to have a positive attitude toward new technologies in general and admit that genetically engineered crop traits ought to be assessed on a case-by-case basis. Nevertheless, they categorically reject adding food products containing GM varieties to their assortments. Thus, the perceived role of biotechnology in creating sustainable agricultural production systems is somewhat ambiguous. Our interview data suggest that the prevalent agricultural sustainability discourse has been largely shaped by consumer attitudes and pressure from strong environmental organizations.

The sensitivity of anti-GM campaigns generally increases as one moves further down in the food supply chain from production to retailing. Among the organizations that participated in our study, LRF (The Federation of Swedish Farmers) appeared to be the least sensitive and the food retailers the most sensitive to anti-GM campaigns. This might be because of differences in their susceptibility to changes in consumer behaviour. It should be noted in this context that consumer behaviour can change rapidly, sometimes overnight, as a result of political campaigns and media coverage. Changes in consumer behaviour have a much more direct impact on food retailers than on farmers.

FARMERS' PERSPECTIVES ON THE ADOPTION OF GM FODDER IN SWEDEN

Although the available GM crops mainly affect farmers' practices and only have limited direct effects on final products directed at consumers, there is a consumer-bias in the literature on attitudes to GMO. The limited farm literature on the topic mainly reports on traditional adoption studies. We expected that farmers, being part of the wider social system, have opinions on GMO that go beyond their running of a farm business. We surveyed Swedish farmers regarding their perceptions of GMOs in livestock feed. In a two-step procedure, an Elastic Net regression approach was combined with Probit regressions to explain their perceptions as a function of their wider farming, social networks and economic characteristics. Results indicate that many farmers are sceptical towards GM feed due to the dominance of multinational companies in the sector, and that they worry about unforeseen consequences of GMO in the environment. Consumer resistance is feared only to a limited extent. Larger farmers and pig farmers are overall more positive to GM feed and expect economic

Our results indicate that many farmers are sceptical towards GM feed due to the dominance of multinational companies in the sector, and that they worry about unforeseen consequences of GMO in the environment. (Photo: Mats Ingvarsson)

benefits, whereas organic farmers and beef farmers are more sceptical. Farmers who have better access to advisory services and are better educated are more positive. However, this group of farmers tends to feel discriminated against in society. In contrast, farmers who engage in direct marketing and rely on frequent participation in community networks tend to be more sceptical of GMO feed, following the general trend in society. Importantly, these results contradict the assumption that farmers only base their views on GMOs on predictions of economic profits or cost savings.

SWEDISH AND DANISH FARMERS' PERCEPTIONS OF GMOS FOR FEED

We have compared how farmers in Sweden and Denmark perceive GMOs in general and GM fodder in particular and what the factors are that shape their perceptions. GM crops are not grown in either of the two countries. However they differ in that Danish, but not Swedish, farmers feed their animals with imported GM fodder. Due to consumer demand, some producers are now labelling their animal products as "GM free", if the animals have not been fed GMO.

We found large differences between the attitudes of Swedish and Danish farmers' with respect to some questions, and similarities with respect to others. 54 per cent of the Danish respondents but only 7 per cent of the Swedish farmers agreed with the statement 'I think genetic engineering gives us new opportunities for developing more hardy crops'. Similarly, 41 per cent of the Danish farmers but only 23 per cent of their Swedish colleagues agreed that 'genetic engineering gives us new opportunities to develop more healthy food'. Of the Swedish respondents 40 per cent concurred with the statement: 'I think genetic engineering is going too far as regards human impact on nature', compared to only 19 per cent of the Danish respondents. However, 38 per cent of the Danish farmers but only 18 per cent of the Swedish ones subscribed to the statement 'I am distrustful of the multinational companies' dominance of genetic engineering'. We propose that the differences between farmers in the two countries may be explained by the fact that intensive pig production has a much larger role in Danish agriculture and that feed import has much larger economic significance.

MARKET POWER IN THE SOY FEED INDUSTRY

We have assessed price transmission in the conventional soybean supply chain originating from Brazil and Argentina and ending at compound feed manufacturers in Sweden and Austria. First we wanted to understand whether the feed industry is competitive enough to pass on the benefits from any upstream cost reductions in soy production to final consumers. And secondly, given that GM soymeal is cheaper, what impact will any upstream cost advantage have on livestock farmers in countries such as Sweden, who currently rely solely on conventional soy? We assessed price transmission elasticity in two stages; when imported soy lands in the EU, and when it is used as an ingredient for compound feed by manufacturers and sold to farmers. The results show asymmetric effects - which means that cost increases get passed on to consumers, but not the cost reductions. This indicates market power in the conventional soy feed industry. In the presence of GM soymeal, substitution effects in Austria and through simulated data in Sweden, suggest strong asymmetric adjustments. This implies that when conventional soy price becomes competitive in South America, livestock farmers stand to lose due to feed industry market power, but more so where GM soy imports are not allowed.

CHALLENGES FOR THE TRACEABILITY OF THE PRODUCTS OF GENE TECHNOLOGIES

Whereas the products of traditional recombinant gene transfer (GMOs) can be detected and identified with laboratory analysis, gene editing (for instance with CRISPR/Cas9) can result in genetic alterations that leave no detectable and/or identifiable traces behind. This means that the outcomes of gene editing are often indistinguishable from the outcomes of natural, spontaneous mutagenesis or from conventional non-directed (random) mutation induction. We have investigated the practical importance of this lack of traceability in six scenarios covering both plant and animal breeding. It is indeed not possible to directly determine or trace site-directed mutagenesis of plants or animals with laboratory analysis. However, it is at least in principle possible to track such changes with pedigree information in combination with statistical information on the likelihood of natural mutations. While pedigree information is available for many animal populations, such written documentation is often not readily or publicly available in plant breeding. Government agencies in countries and regions in which the products of modern gene technologies cannot be used in agriculture, such as the European Union, will have no means of determining whether these restrictions have been complied with in seeds or plant products imported from countries without such restrictions or labelling requirements. Therefore, restrictions against the use of gene technologies in breeding will for certain applications be practically unenforceable in the context of international trade.

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In the EU, the government agencies will have no means of determining whether the restrictions on genome edited crops have been complied with in seeds or plant products imported from countries without such restrictions or labelling requirements.

There is an anecdotal claim that the retailers offer what the consumers demand. However, we found that choices made by retailers had large influence on consumer choices regarding GM food.

Consumers

Consumer attitudes towards biotechnology have an important, arguably decisive, role for both policies and markets. We have studied influences on consumer behaviour, with a particular focus on how the behaviour is affected by the information consumers receive about biotech food products, for instance information about advantages for human health or the environment.

EU CONSUMERS ARE NO MORE ADVERSE TO GM FOOD THAN OTHERS

Previous meta-analyses (studies compiling previous results) of consumer acceptance of GM food have either been limited to studies using monetary measures of preferences, or restricted to certain topics such as media coverage or public perception of genomics. We have performed a meta-analysis covering a wider of consumer evaluations of biotechnologically modified food. We combined the results using different measures of attitudes in a systematic meta-analysis of how consumers evaluate biotechnologically modified food. In contrast to previous meta-analyses, we focused on the questions actually posed to the respondents in each study and used the largest data set that has been meta-analysed with respect to biotechnologically modified food products. We found a negative framing effect for European studies which means that European consumers more often have been approached with questions framed to generate a negative evaluation. When we controlled for the framing effect, we found no support for the common claim that European consumers (on average) have more negative evaluations of food biotechnology than consumers in other parts of the world. Furthermore, there was no support that Swedish consumers are different from the average of other consumers.

This study also showed that food consumers in general, regardless their country of origin, tend to be more influenced by the uncertain risk factors than by potential benefits of biotechnology in food products. Lower price and better taste features build into food products did not affect their attitude significantly, however, food products with medicinal properties were overall viewed as promising.

POLICY AND UPSTREAM ACTORS AFFECT CONSUMERS' RISK PERCEPTION

The European Union has introduced 'opt-outs' rules for biotechnological food products. This allows member states to institute an unlimited, or case-specific, moratorium on commercial release of GM foods within their respective territories and localities. In consequence, regulations may now vary across member states. We wanted to test if the policy context and acceptance by upstream actors influence consumer acceptance of a GM product with direct tangible health benefits. A potato with a low GI was used as example. We conducted a laboratory experiment with Swedish consumers to investigate their perception of how the responsibility for risks related to food biotechnology is distributed among consumers, industry, retail, farmers, and government. We used four scenarios for the regulation of biotechnological products: 1. ban, 2. use only in research and development, 3. imports allowed, and 4. full commercialisation allowed. The subjects considered health risks and environmental risks to be the most important ones. Interestingly, ethical issues were considered important in the scenario with a total ban of the products.

In a second part of the experiment, the subjects were asked to decide whether they would buy/support the GM-potato. But before they made that decision, we presented scenarios to them, which described sequential actions taken by policy makers, farmers, food industry, wholesalers, and retailers. After each such decision, they were also asked how satisfied they were with their decisions. The results we obtained confirm earlier findings that consumers can be segmented into three groups – anti-GM, information searcher, and GM-accepters. The latter two groups made up to about 50 per cent of the respondents. Furthermore, their decisions were influenced by the choices made by other actors in the scenarios, and more so the larger the coherence was among the other actors. Choices made by retailers had the largest influence on consumer choices. Thus we found a causality that goes in the other direction than what is assumed in the anecdotal claim that the demands of consumers direct retailers.

Furthermore, the results from the import and full commercialisation scenarios showed that a majority of Swedish consumers are likely to reject GM food if there is no domestic production, only import. Moreover, the acceptability of GM food for the full commercialization scenario was significantly increased under the mandatory labelling scheme.

Two of the scenarios (research & development; full commercialisation) of the consumer experiment were replicated using a random sample of German consumers. In an additional experiment, we also introduced a second product (GM maize for bioenergy production) to explore the extent to which consumer acceptance and satisfaction depend on product type. In particular, health risks were generally perceived to be lower for bioenergy than food whenever full commercialization was pursued. Furthermore, full commercialization of GM food prompted higher concerns about personal health, whereas use of crops for bioenergy production was broadly related to higher levels of socioeconomic risk. Finally, although the majority of consumers identified health risks as being most important, the consequences for the environment evoked the greatest degree of risk perception. In general, our findings show that the policy regime was the most important determinant for risk perception among the German consumers, followed by the type of risk dimension and level of trust in industry.

COGNITIVE PROCESSING OF BIOTECH INFORMATION

If biotechnological food products are introduced, what will determine people's choice to buy or not to buy such products? Is it determined by previously acquired attitudes, by the information that comes with the product, or by both of these? We performed an eye-tracking study to investigate how the two processes interact when a consumer chooses between biotech and non-biotech products (rape seed oil and potatoes). We found that people with a positive or neutral attitude towards biotechnology made use of a clearly defined biotechnology label throughout the search process when deciding what to buy. For these consumers, the label reinforced the likelihood of choosing the biotechnology product instead of a non-biotech alternative.

CONSUMERS' UNCONSCIOUS DECISION-MAKING

The food products we purchase have a number of labels and much information on the packages, and in the EU all food items that contain one or more ingredients with more than 0.9 per cent GMO have to be labelled. But does the consumer notice labelling and other information on the package, for instance about health effects and environmental aspects? And how is his or her choice as a consumer affected by this information? We performed an eye-tracking study where we found that when people are exposed to positive information in highly noticeable labelling, they are more prone to choose a GM product. Conversely, they can be scared off if negative information about the technology is emphasized. The effect on consumer choice of negative framing is stronger than that of positive framing. However, and most importantly, we found that when people are not presented with a positive or negative information frame, they do not notice the label. In general, people are more “loss averse” than “gain seeking”, and they pay more attention to negative outcomes than to positive ones.

CONSUMERS REACTIONS TO THE TERM "BIOTECHNOLOGY"

How do consumers react to the term "biotechnology"? We used a fictive example with packaging of fish, labelled as; wild-caught, farmed, sterilized with hormones, sterilized with pressure treatment of newly fertilized eggs, and so on, and observed how the consumers’ willingness to buy was affected. We found that labelling of genetically modified fish can deter consumers from buying it. For some of the participants, it was enough if the label said "biotechnology" without any explanation, to make them choose a wild-caught fish instead. If the term "biotechnology" was included for the fish from pressure treated eggs, then the positive view of wild-caught fish was strengthened (although it was considerably more expensive). However, an even stronger negative effect on consumer choice was obtained for fish labelled as hormone treated. (Hormone treatment and pressurization of eggs are alternative ways to sterilize fish.)

THE RELATIONSHIP BETWEEN PERCEIVED RISKS AND PERCEIVED BENEFITS

Previous research has shown an inverse relationship between perceived risks and perceived benefits. In other words, people who consider the risks with an activity to be high have a tendency to consider the benefits to be small, whereas those who consider a risk to be low tend to consider the benefits to be high. We investigated the extent to which a person’s dominant thinking styles can predict to what extent she exhibits this tendency concerning potential hazards related to medicine and food. We used the psychometric Need for Cognition Scale (NFC). A high score on this scale is interpreted as indicating a rational mode of thinking. In a representative sample of 3228 Swedes, we found that those with a high NFC score had the mentioned tendency, which means a negative correlation between perceived risks and perceived benefits. However, those with a low NFC score had the opposite tendency, which means a positive correlation between perceived risks and perceived benefits. These results indicate that there are important connections between a person's general thought style and her perception of risks and benefits.

EFFECTS OF INFORMATION ON GMO ON ATTITUDES AND PURCHASES INTENTIONS

In previous studies, information about benefits of GMOs did not lead to attitude change among those who were initially opposed to such products. We investigated the effects of information that has been adjusted to the individual’s initial perception of risks and benefits. Based on subjects’ on prior risk-benefit perceptions of gene technology for plant breeding, we provided them with information on advantages with GM potato. The results show that persuasive information matching the individual’s initial risk perception led to more favourable attitudes, not only among those who’s initial attitude was positive to the technology but also for those who were initially strongly against gene technology. The effectiveness of the communication was also found not only in attitudes but also in purchases intentions. Furthermore, we found that the health implications of GM potatoes played the most important role in inducing changes in purchases intentions. This confirms other recent research showing consumers’ attitudes to GM food to be dominated by concerns of health implications.

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The tomato Flavr Savr was the first GM product to be sold as food (in 1994). (Photo: Donald Grierson)

Media

The first GM food products emerged on the market in the 1990s and several scientific breakthroughs were made public. The public debate on GMO really took off in the late 1990s. Events like the launch of the human genome project in 1990, the cloning of the first sheep, Dolly, in 1996, and the decoding of the first human chromosome in 1999 received considerable media attention. Several influential reports were also published during this period, reviewing the scientific evidence and ethical issues emerging around the possibility of genetically modifying living organisms. How has this reflected in media?

THE SWEDISH MEDIA DEBATE ON GMO 1994-2017

What did the largest Swedish newspapers, evening papers and farm magazines write about GMO? We did a semi-quantitative content analysis where we categorised all articles about GMO and food or farming published in the four largest Swedish newspapers and four largest evening papers and the three largest farm magazines (based on readership) from 1 January 1994 to 2 January 2018. Each article was read and categorised according to a survey designed by us, asking questions about topics discussed, geographic focus and types of modifications and organisms etc. We found that that in the 1990s the debate on GMO in Sweden, was focused on consumer issues including health risks and labelling, underpinned by the concern that the governing bodies were not in control or not taking sufficient responsibility. Over time, the topics discussed in the media broadened, although labelling, health risks and food remained frequently reported topics. Environmental issues seemed to have made less of an impact on the Swedish debate than in most of Europe. The narrow focus on consumer aspects in the media contrasts with findings in other studies, according to which the Swedish general public has more general concerns, including ethical aspects, unforeseen consequences for the environment, the perceived unnaturalness of GMOs and their connections with multinational companies.

The geographical focus in media reporting also shifted over time. There was a strong focus on the EU in the early years of the debate, but more attention has been paid to Sweden in later years. Whereas the academic literature and the wider international debate on GMO have focused on agriculture in the Global South along with issues of food security, poverty and farmers' rights, our study indicates that these issues were almost completely absent in the Swedish media. In the beginning of the studied period, researchers did not participate much in the media debate. However, when they became involved (in 2012), they seem to have had an impact on the debate, which has becoming more positive to GMO. In contrast, the general public does not yet appear to have shifted towards a more positive view of GMO. It remains to be seen whether the media will lead the way in changing public opinion.

TESTING FOR STRUCTURAL BREAKS IN OBSERVED MEDIA DISCOURSES ON GMO IN SWEDEN

How did the public discourse in Sweden, and specifically within the farming community, concerning GMO in food and agriculture shift in intensity and content from 1994 to the end of 2017? And how were these shifts connected with and potentially influenced by other ongoing debates and events in society? And to which extent can the structure of the debate in the agricultural community be seen as a separate discourse from that of the general public?

We present a quantitative discourse analysis of articles published from 1994 to 2018 in Sweden's daily and evening newspapers and farming publications about genetically modified organisms (GMO) in food and agriculture. A combination of content analysis and statistical simulation techniques was used to identify structural breaks in the dataset to contribute understanding about which topics influenced the direction of debates over time. The results show that the general discourses on GMO were heavily influenced by consumer issues, farmers' perspectives and risk, regulation and trade. Farming publications were influenced more significantly by discussions about patents and intellectual property rights. Surprisingly, food security, which has been hotly debated in relation to GMO in other contexts, was not shown to substantially affect media discourses in Sweden.

MEDIA REPORTING ON GMOS – A CROSS-ATLANTIC ANALYSIS

The differences between the GMO legislations in the USA and Europe have been controversial for decades. Although there is broad scientific coverage of possible causes for this divergence, little is known about the role that popular media play in the related political discourse. We compared the coverage of biotechnology from 2011 to 2013 in two leading newspapers, The Washington Post in the US and The Guardian in Britain. The two newspapers differed in their intensity of reporting on GMO issues, but were alike in terms or content. On both sides of the Atlantic, the central actors were scientists and NGOs, arguing mostly about the use of GMO in the agricultural sector. In both newspapers the debate appeared to be locked in a stalemate of potential risks against potential benefits, with neither of the two positions clearly dominating the discourse.

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Although debated in relation to GMOs, food security, does not seem to substantially affect mediated discourses in Sweden.

Almost everyone wants to eat "natural" rather than "unnatural" food. But what does it mean that food is natural?

Ethics

Traditionally, the discussion on the ethics of technology, including that of biotechnology, has been characterized by diverging views on whether technology in general, or some specific type of technology, is benign, or a threat. In particular within environmental ethics, where most of the academic debate on the ethics of agricultural biotechnology has been carried out, attitudes towards new technologies has tended to be hesitant, sceptical, or even hostile. Even among technology optimists, there is recognition of a need for assessment and management of technology and its consequences. This discussion contains elements that are highly relevant for the regulation of agricultural biotechnology.

PLAYING GOD AND THE HUBRIS ARGUMENT

The old notion of hubris, given to us by the ancient Greeks, has been used by several critics to characterize agricultural technology. This study takes as starting-point the argumentation put forward by Ronald Sandler, who offers us an understanding of hubris, which in his view gives us a prima facie reason not to use GM crops. Basically, he argues that biotechnology follows the tradition of manipulating and dominating our environment. Since this tradition has caused many of our current problems, he maintains that it would be hubris to rely on further manipulation and domination in the form of GMO. Our study concludes that Sandler's hubris argument fails for several reasons:
1. He fails to have a proper understanding of agriculture as an inherently technological practice that is radically different from "nature"; 2. the notions of control and manipulation that are central to his concept of hubris are unclear and it is not obvious how they can be applied to agriculture; 3. There are many types of GM crops, and some are profoundly different from others in aspects that are relevant for this argument. 4. Even if we accept Sandler's argument about hubris, it actually has no impact on the moral status of different GM crops.

NATURALNESS AND FOOD LABELLING

Almost everyone wants to eat "natural" rather than "unnatural" food. But what does it mean that food is natural? The common cereals are products of ten thousand years of plant breeding, and most of them are so different from their wild relatives that they cannot survive without the farmer's help. Is "natural" just a label we put on food that we are accustomed to, or does it have a more substantial meaning? This is an important question for agricultural biotechnology, not least since food is sometimes labelled with claims such as "all natural ingredients". There is controversy on how to justify, design, and implement such labelling without misleading the consumer. We argue that naturalness is not one single concept, but several ones (polysemy). Furthermore, those concepts typically allow degrees, so that things can be more or less natural. This complexity should be reflected when food manufacturers label their products. In consequence, labelling for naturalness should provide multi-dimensional information. One way to do this is to use a small number of distinctive labels, corresponding to different aspects of naturalness.

PRECAUTIONARY LEGISLATION IN NEED OF AN UPDATE

The precautionary principle has its origin in international treaties on the environment from the 1990s, in particular the Rio Declaration from 1992. It has been incorporated into European legislation as well as the legislation of member countries such as Sweden. Although there are minor variations in its formulation, the principle has very much the same meaning in the different official documents. It says that measures can or should be taken against a potential threat to the environment or human health, even in cases of uncertainty, i.e. even in the absence of full scientific evidence that the danger is real.

We provide a proposal for how the precautionary principle can be applied regarding GMOs. Its basic idea is that precautionary measures to protect human health and the environment should be science-based. This means that for precaution to be applied there should be scientifically credible evidence of a potential danger. On the one hand, this evidence need not be conclusive, i.e. precaution can be based on scientifically credible suspicions of danger. On the other hand, precaution should not be based on guesses that have no scientific support. Furthermore, precautionary measures should be updated as more scientific information becomes available. Decision makers should be prepared to strengthen the precautionary measures if the danger turns out to be greater than initially suspected, and to reduce or lift them, should the danger prove to be smaller.

Most current legislation on agricultural biotechnology has not been scientifically updated in this respect for several decades. It therefore reflects outdated criteria for identifying products that can cause problems. Modern knowledge in genetics, plant biology, and ecology has provided us with much better criteria for identifying the potentially problematic breeding projects at which precautionary measures should be directed. Legislation on agricultural biotechnology should be scientifically updated so that it makes use of the scientific information that is available today.

THE PRECAUTIONARY PRINCIPLE AS A MID-LEVEL PRINCIPLE

We present a new way of conceiving the precautionary principle, namely as a mid-level moral principle. This is a type of moral principle that has considerable application in medical ethics. Mid-level principles do not offer any ultimate justification of moral judgments. Instead they are more flexible and must be balanced against other principles. They can serve as general guidelines from which more specific rules are formulated. A tentative version of a mid-level precautionary principle is presented: 'Reasonable precautionary measures should be taken to safeguard against uncertain but non-negligible threats'.

ETHICAL PRINCIPLES FOR PLANT BREEDING

The ethical discussion on plant breeding has been almost exclusively focused on a discussion on GMOs. Unfortunately, this discussion has not been sufficiently informed by modern scientific knowledge, and it does not have much contact with the actual activities of plant breeders. We attempt to provide a framework for a broader and more practically oriented ethics of plant breeding. It puts much focus on plant traits and on breeding goals, which are also central concepts in the day-to-day work of plant breeders. The following aspects are pointed out as central for ethical discussions:

- The selection of breeding goals, a choice that should be guided by the long-term public good and by the interests of smallholders and consumers,
- The implementation of these breeding goals in a system for plant breeding that is currently dominated by large international companies with other priorities,
- The measures that plant breeders need to take in order to prevent the inadvertent release of cultivars with undesired traits, such as toxicity or weediness,
- How the (science-based) precautionary principle should be applied to plant breeding8.
- How a food labelling system can ensure adequate information to consumers wishing to avoid foodstuffs obtained with biotechnology, without contributing to unjustified worries concerning these products, and
- How the intellectual property regime can be reformed and/or better applied, in order to empower subsistence farmers and better support innovations that are important for sustainability and food security.

EVALUATING ETHICAL TOOLS

Ethical tools are devices for facilitating deliberation, reasoning, and decision-making about ethical issues.

They might, for instance, consist of computer games that help the users to clarify their own ethical standpoints or of meeting formats that help a group of decision makers to arrive at an ethically well-founded decision. There are a sizable number of such tools available. Here we propose a criterion for the evaluation of such tools: They should be evaluated in terms of their purposiveness, i.e., how well they achieve their intended purpose(s). Just like regular tools, such as hammers, shovels, and scalpels, a good ethical tool is one that fulfils its purpose. To make this more concrete, a categorization of ethical tools is proposed, and assessment criteria for each of the categories are provided:

1. All ethical tools can be judged on the crucial qualities of comprehensiveness, or how well a tool includes all relevant considerations, and user-friendliness.
2. For tools that have the goal of reaching a decision in a democratic context, the criteria of transparency, action guidance, and justification of decision-supporting mechanisms are important.
3. For tools with the aim of engaging the public, procedural fairness is crucial. Procedural fairness ensures that the use of the tool, if used by a group, is carried out in a fair and justified way. Several such tools have been used in the assessment of biotechnology in Europe.

BREEDING OF FARM ANIMALS WITH GM AND GENE EDITING

Genetic modification and especially genome editing has opened new opportunities for applications in farm animals. Especially for larger breeding programs this raise new questions, both practical and ethical ones. Values and views on the integrity of animals, naturalness and risk assessments are other factors that affect how the new technologies could be assessed and received in society. Can we, do we need to, and should we, use these techniques in commercial breeding? Does it matter what reproduction technology we use, how we handle cows and embryos, or what traits we change? Are there alternatives ways to reach the same goals? We looked into two cases of biotechnology in livestock breeding: genome editing with the example of polled cattle and GM with the example of udder health in cattle. Because similar biotechnological methods are required to introduce a modified embryo into a surrogate female animal, the technologies are in practice quite similar. This should have an impact on the ethical discussion. The techniques can be used to different extents and for different purposes, which will determine to which degree the animals are affected. The new techniques should be evaluated within the breeding context they are to be applied in, before being used on a larger scale. Animal breeding organizations should consider setting up ethical committees to penetrate possible ethical issues when applying new technologies in the future. The committees could include researchers, industry representatives, representatives of the public, as well as ethically or philosophically educated persons.

How can a food labelling system ensure adequate information to consumers wishing to avoid GM foodstuffs without contributing to unjustified worries concerning these products?

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The current market structure does not give farmers sufficient incentives to produce more healthy products. Farmers are often paid for quantity, without any reward for health-related quality. The food production chain needs to be reformed so that consumer demands for healthier products have a stronger impact. (Photo: Alexander Mahmoud)

Breeding for public health

Significant improvements in public health can be achieved if we manage to enhance the nutritional quality of our diets. This is true for populations in all parts of the world, although the needs for dietary change are partly different. Plant and animal breeding can contribute to promote human health by providing new and healthier food products that farmers can produce in an economically viable way and consumers can choose to buy and eat. However, this will only be achieved if breeding makes full use of knowledge about nutrition, consumer behaviour, farming and agricultural economics. A strategy is needed for breeding for public health. We present a strategy for plant and animal breeding for public health, developed by a multidisciplinary group of researchers, representing food science, nutrition science, animal and plant breeding, behavioural science and ethics. The strategy includes the following key conclusions:

- Breeding for health-promoting traits should target both micronutrient deficiency (primarily low-income countries) and diseases such as overweight and coronary disease (primarily high-income countries).
- The highest priority should be given to healthy variants of traditional food items that can be introduced universally i.e. completely replace older, less healthy variants, followed by products that may not be suitable for universal introduction but have a large market potential in major consumer segments.
- Efficient incentives to produce healthier foodstuffs are currently lacking and need to be created. The crops and products must be attractive for farmers to produce and for consumers to eat.
- Social influence through general endorsement is key to obtaining a general adoption of more healthy food habits among consumers.
- The best available technologies should be used. For certain crops and traits, modern technologies such as genetic modification and genome editing will be needed.
- Breeding has to be combined with economic development, treatment of diseases that aggravate malnutrition, and adequate dietary information.
- The current market structure for farm products does not give farmers sufficient incentives to produce more healthy plant and animal products. Farmers are often paid for quantity, without any reward for health-related quality. The food production chain needs to be reformed so that consumer demands for healthier products have a stronger impact.

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Shifting from import of non-genetically modified soy to GM soy in Sweden would imply an increased negative environmental impact since the cheaper imported GM soybean would replace our domestic production of protein feed.

Environmental impacts

From field to fork, food production and consumption have environmental effects. Global warming, freshwater ecotoxicity, acidification, eutrophication and losses in biodiversity are some examples of agricultural impact on the environment. The level of the impact depends on the cropping systems used, agricultural methods, crop traits, livestock characteristics as well as transport distances and waste after harvest. There is little evidence that the effects of genetically modified traits on ecosystem processes act with different mechanisms from those of traits modified using conventional breeding methods. Still, segregated supply lines, labeling requirements and other indirect consequences of farming with genetically modified organisms make it possible to distinguish between the effects of GM and non-GM crops.

GENETICALLY MODIFIED ORGANISMS INFLUENCE AGRO-ECOSYSTEMS INDIRECTLY

We have reviewed the scientific literature on the environmental effects of genetically modified organisms (GMOs), with a particular focus on how genetically modified plants, animals and microorganisms can affect agricultural production, nutrient cycling, greenhouse gas fluxes, biodiversity and trophic interactions (who eats whom). One of our major conclusion is that when GMOs have affected agricultural ecosystems, then that has been through indirect effects rather than direct impact. That is, it has not been the modified trait of the crop itself that has been observed to affect the environment. Instead, the cultivation of specific GM crops has for instance resulted in increased use of a certain weed control agent, or it has changed the tillage management, and this in turn has had effects on the environment. New GMOs with new traits might influence ecosystems directly, but in order to find out, extended studies on these traits are required. We emphasized that both GM and non-GM crops can have traits with (direct or indirect) effects on the environment.

ENVIRONMENTAL CONSEQUENCES OF INTRODUCING GM SOY FEED IN SWEDEN

How would it affect the environment if Swedish farmers began to give their animals feed based on imported GM soy, which is common in many other European countries, instead of using only GM-free soy as Sweden does today? Through a life cycle analysis we show that there are no significant environmental gains from importing non-GM soy, instead of GM soy, when comparing the two parallel supply lines. However, the cheaper GM based feed would lead to increased demand for soy, which could lead to deforestation in South America to release new farmland. At the same time, the demand for Swedish protein crops would decrease. The most environmentally friendly option would be to use plant breeding to develop protein-rich plants that can grow in the Nordic climate, and then use them as animal feed to replace soy that is now being imported.

PROMOTING BIODIVERSITY IN AGRICULTURAL LAND DESPITE BIG AGRICULTURE

Covering about half of the EU's land area, agricultural land provides important habitat for many European species. This biodiversity can be threatened by monoculture, high intensity farming and extensive pesticide use. We have examined opportunities and challenges for promoting more biodiversity-friendly agriculture in the EU. In view of the fact that several studies show that organic agriculture is better placed to promote biodiversity we have studied the difficulties in promoting organic farming alongside other forms of agriculture, especially farming with GM crops. The difficulties in solving coexistence problems are partly linked to the requirements for product labelling that prevail in each system. In light of the coexistence problems between different agricultural forms, we suggest that biodiversity should be a consideration in substantive requirements for examinations in the pre-market phase, and that labelling requirements should to a greater extent mirror agricultural methods/techniques beneficial for biodiversity.

If we ignore other concerns (ethical, religious, etc.) against GM agriculture than those associated with biodiversity, then GM crops would have to be assessed according to their traits. This requires plant breeders to develop GM seed, which, in addition to increased productivity, provides a long-term environmental benefit. Then it should be difficult to argue that GM crops with herbicide resistance qualify, because this trait is associated with harms to biodiversity, as well as other environmental harms. But if for instance the GM seeds require less pesticides, or reduce the need for fertilizers, then they may be positive for biodiversity. This presupposes that these biodiversity-positive GMOs are perceived as compatible with organic farming. At least, to the extent that objections to GM farming are primarily based on environmental harm, the acceptance of GMO involvement is likely to depend on whether agribusiness can show the benefits of GM crops for biodiversity. So far, efforts in this direction have not been striking.

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GM crops that are positive for biodiversity should be perceived as compatible with organic farming.

Policy and legislation

In most legislations around the world, the introduction of GM crops requires an extensive risk assessment, based on field trials and other experimental studies. The legislation that regulates genetically modified organisms (GMOs) in Sweden is essentially an implementation of decisions made on the European level. Europe differs from most other parts of the world in two important ways: Very few GM crops are allowed to be cultivated, and special labelling is required on foodstuff containing GM material (more than 0.9 per cent of each ingredients). As a consequence of this, very few foodstuffs containing GMOs have found their way into the European market, whereas for instance in Northern America they are very common.

The current legislation in EU refers to GMO as "an organism in which the genetic material has been altered in a way that does not occur naturally through fertilisation and/or natural recombination" (Directive 2001/18/EC). The legislation on GMO is process-based, which means that it is the technology used by the breeder, not the traits of the plants that determines whether it will be subject to the restrictions of this legislation. The present EU legislation is more than 25 years old, and during those years genetic engineering has evolved rapidly. Based on a recent decision by the EU court, gene editing technologies, which were unknown when the legislation was adopted, are treated in the same way as traditional genetic modification (gene transfer).

On April 2, 2015, a new EU legislation came in force, which allows Member States to ban or restrict the cultivation of GM crops in their territory. Such a national decision has to be based on policy objectives not conflicting with the assessment on which the Union based its approval. Such objectives may refer to environmental policy, land use, town and country planning, socio-economic impacts, and coexistence between different forms of agriculture. Most EU countries have decided to activate this "opt-out" mechanism in one way or other but Sweden has decided not to do so.

Crops domesticated and bred through different methods and technologies. Unintentional selection breeding started the domestication process of our crops over 10 000 years ago. With increased understanding of heritability, the structure and function of DNA, and a parallel technical development, the possibility of making more targeted changes has increased. Compared to selection breeding and hybridization, newer methods (especially gene transfer and genome editing) are much faster processes.

	Selection breeding	Hybridization (of species)	Mutation breeding (radiation/chemical)	Genetic modification (gene transfer)	Genome editing (without gene transfer)
Number of genes affected	10 000 - 300 000[1]	10 000 - 300 000[1]	300 – 700 000[1]	1-3[2]	1-3[2]
Tests for effects on humans and the environment are required	X	X	X	✓	✓[3]
Needs to be labelled[3]	X	X	X	✓	✓[3]
Traceable	X	✓	X	✓	X
Allowed in organic farming	✓	✓	X[5]	X	X
Examples	Most plants we eat	Apples, wheat, rice, triticale, oilseed rape	Pear, grapefruit, oilseed rape, barley	Corn, oilseed rape, aubergine, soy, papaya	Champignon, apple, soy, oilseed rape, potato

1. Depending on species. 2. More genes can be affected. 3. In the EU. Several other countries do not regard those crops as GMO. 4. In the EU, products in which an ingredient contains more than 0.9 per cent GMO needs to be labelled. 5. According to the IFOAM, mutation breeding is not allowed but in practice, this cannot be controlled for and many of those crops are grown also on organic farms. Sources: Sikora et al. 2011. Int J Plant Genom doi:10.1155/2011/314829, Genetic Literacy Project.

A STUDY OF THE ORIGIN OF THE EUROPEAN GMO LEGISLATION

To guide future potential changes in the EU GMO regulatory framework, we have analysed the early drafts from the late 1980s when the first GMO Directive was developed. It turns out that certain crucial concepts that were initially envisaged have failed to materialise in practice and/or in actual legal text. These concepts include 1. a later shift in focus from technique to the organisms and their traits, 2. a periodical updating of the technical annexes of the legislation, as technology advances, 3. the acknowledgment of a history of safe use, as experienced is gained in Europe or elsewhere, and 4. the acknowledgement of benefits that may result from the use of GM technology. We propose that these issues should be reconsidered in future reforms of the legislation.

ANALYSIS OF THE EU REGULATORY FRAMEWORK FOR GMOS

We have analysed several aspects of the European legislation for GMOs in a cross-Atlantic collaboration with a team from the US, we showed that the current regulations essentially serve as barriers to the market, in particular for less resource-strong actors such as small- and medium-sized companies and plant breeders working at universities. We have also contributed to the discussions on regulatory developments, such as those arising from the emergence of novel molecular technologies that allow a continuum of genetic alterations in organisms in addition to the older transgenic approaches. Since the concept of naturalness is crucial for the legal definition of a GMO, we have provided a number of key considerations for the evaluation of whether or not a certain genetic alteration can be said to occur naturally.

- Does the alteration occur in nature?
- What is the likeliness for the alteration to occur?
- Is it an alteration beyond what does occur naturally by mating and/or natural recombination?

We point out the inconsistency, from a risk assessment point of view, of a legislation that applies strict regulations to the products of precise and specific genetic alterations, whereas the products of random genetic alterations will not be subject to this type of regulatory provisions. Given the high societal interest in GM technologies together with the EU Responsible Research and Innovation (RRI) approach, we also investigated the level of balance in stakeholder input in the decision-making procedure and novel legislation development, and found that the balance may be biased against science in various case examples. The three main examples of this are regulatory delays for GM maize, the opt-out directive, and the lack of regulatory status for new plant breeding technologies.

WEAKNESSES IN THE EU LEGISLATION ON CROP CULTIVATION

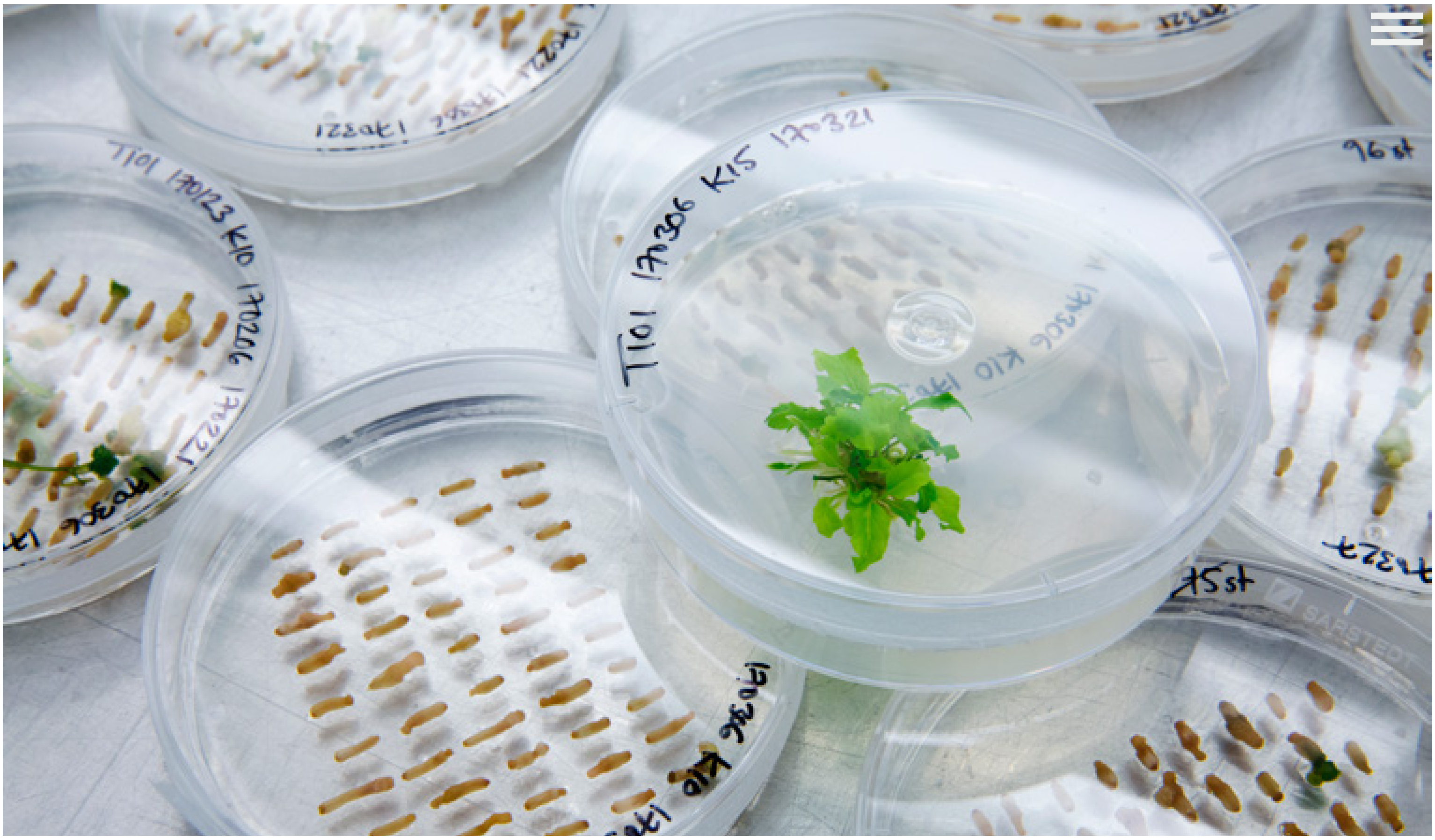
We have analysed the Directive 2001/18/EC on the deliberate release of GMOs into the environment with the help of four questions that concern the quality of the legislation. We asked: Is the legislation predictable? Does it include discrimination/equal treatment? Is it proportional to the risks? Does it take the recent science into account?

We found shortcomings in the legal certainty and clarity of the legislation. For instance, if new scientific evidence emerges showing that a GM crop is less risky than previously thought, then it is not possible to change the assessment. If, on the other hand, the risk is greater than what was believed when authorization was given, then further precautions may be required. However, it was more difficult to determine if the GMO legislation is proportionate, that is, if it is reasonable comprehensive and has the right level of stringency. Proportionality depends on the purpose of the legislation, which in turn reflects political values. If the GMO legislation only existed for the purpose of minimizing the risks and protecting the environment and health of humans, then criticism about disproportionality is justified, as GM crops that obviously involve minimal risk are covered by the rules. However, legislators may also take other considerations into account.

In conclusion, we suggest that different crop varieties could be regulated using different levels of risk assessment, depending on how probable it is that they will pose a risk. Another option is a framework that regulates all new varieties of crops based on their performance from the perspective of sustainability, not considering what breeding technology that have been used in the development.

CHALLENGES TO THE AUTHORIZATION OF GENE EDITED PLANTS

We have shown practical and legal problems with the authorization of gene editing products in the EU, according to the dominant interpretation of the legislation and the judgment by the Court of Justice of the European Union in the case C-528/16. This study focuses on products of gene editing featuring single nucleotide variants, and shows the difficulties with developing an event specific detection method or even filing a complete application for authorization. We show that the current treatment of such products as



A good case can be made for a precautionary approach to risks, such as invasiveness. However, how the traits have been obtained is irrelevant from a risk perspective. We conclude that the legislation is inconsistent from the perspective of risk assessment and risk management.

GMOs requiring authorization leads to legally unacceptable consequences, such as the violation of principle of proportionality, and propose changes in the application of the legislation.

EFFECTS OF THE EUROPEAN LEGISLATION ON INNOVATION THROUGH PLANT BREEDING

We have studied the potential for the development of rapeseed and rapeseed derived products through analysing the dynamics of changes in three factors – cultivation area, volumes of production, and intellectual property rights (patents and plant variety rights). Poland and Germany were chosen for the comparison, due to their relatively high share in the rapeseed market and their similar conditions. The results indicate a growing trend in the innovative activity regarding rapeseed and rapeseed related products in the compared countries. An analysis of the current GMO legislation indicates that it may constitute an obstacle to that activity, through creation of a hostile environment for the commercialization of its products.

INTRODUCTION OF NEW CULTIVARS – A HYPOTHETICAL RISK-BENEFIT ASSESSMENT

In order to analyse the consequences for plant breeding of the European GMO legislation, we used four hypothetical introductions of new cultivars as examples: two varieties of field cress and two varieties of potato, with one of each species being classified as GMO. All four cases are realistic since they are based on ongoing plant breeding projects at SLU. In all four cases there are potential risks that have to be eliminated in the course of plant breeding. For instance, invasiveness depends on the crop's ability to survive outside of an agricultural field, which could be a risk factor in field cress. It is also well known that conventional potato breeding can lead to inadvertent increase in toxic substances such as solanine. Thus a good case can be made for a precautionary approach to those risks, however, how these traits have been obtained is irrelevant from a risk perspective.

Based on these comparisons, we conclude that the legislation is inconsistent from the perspective of risk assessment and risk management. Another problem is that, contrary to most other risk-related legislations, it is based on assessments that only considers risks, and not the associated benefits. Usually risks are required to be weighed against the benefits.

PLANT BIOTECHNOLOGY FROM A SCANDINAVIAN PERSPECTIVE

We have performed a comprehensive review of the status of plant biotechnology in the Scandinavian countries Sweden, Denmark, Norway and Finland. This region is unique in terms of climate, temperature and day length, but also due to its specific regulations on the use of fertilizers and pesticides. Crop varieties, adapted to the region, have been developed both within government-funded programmes and by smaller breeding companies. The current global trend, with plant breeding performed by just a few large multinational companies, leads to a strong focus on a few crop varieties developed for large markets. This poses a challenge for the development of crops well adapted to the unique Scandinavian climate. The relatively small markets in Scandinavia are a limiting factor for private investment in regionally adapted plant breeding. We show that public investments in crop breeding for this region is of outmost importance for us to achieve a sustainable agriculture with reduced negative environmental impact, such as reducing the use of pesticides and an efficient use of fertilizers.

NATIONAL PERSPECTIVES ON THE REGULATION OF SITE-DIRECTED MUTAGENESIS

We have reviewed the policies taken by Sweden regarding site-directed mutagenesis, and found that it aligns with those taken by several other EU members, and that it also coheres with the scientific consensus. In contrast the EU regulation applies rules to this technology that were decided before the technology existed. Consequently, these rules are not based on a science-based risk assessment of site-directed mutagenesis. To put the EU policies and regulations for site-directed mutagenesis into an international perspective, we compared it with countries that have already established, or are in the process of establishing, regulatory provisions for these technologies. These countries include Argentina, Australia, Brazil, Canada, Chile, Colombia and the USA (USDA). The EU approach differs radically from these other countries, which may have considerable implications for international trade.

ANALYSIS OF A PROPOSAL FOR AN UPDATED GMO FRAMEWORK

The Dutch Ministry of Infrastructure and the Environment has proposed that the EU amend the Directive on deliberate release of GMOs into the environment. According to the proposal, plants that have been developed with genetic engineering, but at the end do not contain any new DNA sequence and have not received any genetic material beyond what they could get through traditional plant breeding methods, do not need to be regulated as GMOs. This would mean that gene editing can be used in plant breeding. We describe a number of publicly funded projects in several European counties, which use this technology to provide healthier crops and crops that are better for the environment due to, for example, less need for insecticides. We also mention examples of how the technology can be used to obtain plants to be used a greener production of pharmaceuticals and substances for industrial purposes. We conclude that the proposal would open up important new possibilities for a sustainable agriculture in Europe.

AN OPT-IN MECHANISM FOR EUROPEAN COUNTRIES WISHING TO GROW GM CROPS

Since 2015, the EU legislation states allows Member States to prohibit the cultivation of GM crops within their own borders, even if the crop has been approved for cultivation at EU level. Seventeen member countries (including Germany, Poland, France and Italy), and two regions (Wallonia in Belgium and Scotland, Wales and Northern Ireland) have chosen to take advantage of this so-called opt-out mechanism and prohibited the cultivation of the currently only GM crop approved for cultivation within the EU, a GM maize, in their own territories. This maize, approved in 1998, carries a gene from the soil bacterium *Bacillus thuringiensis*, which produces a protein toxic to specific insect orders. This makes the plant resistant to the most severe insect pests.

In cooperation with colleagues from other EU countries, we present detailed proposals for opt-in mechanisms that would allow countries that wish to cultivate GM crops the opportunity to do so, provided that the crops in question have first been assessed as being as safe as a conventional counterpart by the European Food Safety Authority (EFSA). According to one proposal, the opt-in mechanism could come into force immediately after EFSA's safety assessment. According to a second proposal the mechanism could be applied later in the process, after the Member States have voted on whether or not to approve the safety-assessed GM crop for cultivation. In the latter case the opt-in mechanism would come into force only when the member states fail to make a decision for or against the cultivation.

A DEFINITION OF TECHNOLOGY NEUTRALITY

Technology neutrality has often been emphasized as a desirable feature of the regulations surrounding agricultural biotechnology. Roughly, it means that the properties of a product, rather than the technology with which it was obtain, should determine its regulatory status. However, the concept is not well-defined. We argue that technology neutrality must be understood in relation to the regulatory rationale, that is, the purpose of the regulation. This means that technology neutrality stating is defined as meaning that similar rules apply to technologies that are alike in terms of the regulatory rationale. For instance, if the reason for regulating some technology is safety, then the regulation is technology neutral to the extent that it treats equally safe technologies in the same way. Technology neutrality can be viewed as coming in degrees. The current GMO legislation on in the EU can be described as process based and technology specific, since it singles out crops developed by the use of a certain technology and subjects them to more stringent regulation than other crops, while the USA and Canada among other countries have GMO legislations that are described as product based and more technology neutral.

REFORM OPTIONS FOR THE EU GMO LEGISLATION

The current legal framework has been criticized for hindering innovations that would be beneficial for health and environment. In order to pave the way for reforms that would make such innovations possible, an international group initiated from Mistra Biotech analysed various aspects of the legislation that may be subject to changes, and sorted them into three main categories: 1. scope and definitions, 2. risk governance (assessment and management), and 3. post-authorization procedures (labelling, co-existence). We believe that the reforms, if implemented, should help to make the EU legislative framework for GMO more predictable and innovation-friendly. The political reality is also taken into account, and a particular emphasis is put on reforms that would seem to be possible to achieve within the near future. We favour a more graded approach that does not treat all GM crops alike, and we also emphasize reform options that increase the scope for decisions on a national level.

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Advances in animal breeding

While farm animals have traditionally been selectively bred to increase the production of animal proteins like milk, meat and eggs, the current breeding goal is shifting more towards animal health and their ability to tolerate stress. Genomic selection is the most modern selection method in animal breeding today. It saves time and reduces costs compared to methods based only on phenotyping, the measurement of individuals' traits and characteristics. Phenotyping takes a lot of time, and many traits can only be monitored in adult animals. With genomic selection the phenotypes can instead be predicted by doing DNA-tests, and breeding choices can be based on genetic markers. Information on the protein level, proteomics, can be used in the same way. We have focused on improving the genomic selection method and analysed the potential to use proteomic information as an alternative or complement in livestock breeding. We have also looked into the possibility to use genome editing in breeding of farm animals.

In both animal and plant breeding, the main goal is to select individuals that will improve future generations with respect to desired traits. For that purpose, statistical methods are applied to predict the genetic values of the individuals, and to rank the candidate progenitors of the future generation. In the last decade, genomic selection, a selection method based on the availability of hundreds of thousands of DNA markers, has gained popularity. Today this method is applied routinely for selection in several livestock species like dairy cattle, pig, chicken and beef cattle. Nonetheless, the applications of strategies based on genomic selection are not always straightforward for all species and populations, and studies are needed to determine the specific requirements for each case.

During the eight years of Mistra Biotech, genomic selection has brought about a paradigm shift in livestock and plant breeding. In livestock, the changes have been most paramount for dairy cattle breeding where the whole approach to breeding and the structure of the breeding population have been turned the other way around. Previously, many young bulls were test-mated to at least 100 cows. Only after their daughters had completed their first full lactation was it decided which bulls would be certified for breeding. With the extensive uptake of genomic selection, the current breeding bulls are selected shortly after birth (some even as embryos) purely on the basis of their DNA profile. They can therefore start their career as breeding bulls as soon as they are fertile. This has more than doubled the genetic progress for many traits, in particular those that have a lower heritability, such as fertility and disease resistance. However, genomic selection has also increased the rate of inbreeding, and the challenges of bull fertility have become more important because bulls are used at a much younger age.

CONTRIBUTIONS TO A GLOBAL PROJECT

The 1000 Bull Genomes Consortium consists of 40 partners over the world who work to assemble whole genome sequences of cattle from around the world, to provide an extended data-base on genomic information. We have contributed valuable genome information on Swedish dairy bulls of the Swedish Red White Cattle (SRB) breed, and we have gained access to the full data from the consortium which now has whole genome information from more than 1700 animals. We use the 1000 bull genomes as a reference to improve the DNA information on the animals in our genome mapping studies. Ongoing projects have been able to capitalize on this further in comparing the DNA of old bulls (from the archives) to that of modern bulls.

INCLUDING PRIOR KNOWLEDGE IN GENOMIC SELECTION

The aim of genomic selection is to predict the genetic merit of an individual based on genomic marker information. The most common statistical method used to predict the genomic breeding values (GBLUP) assumes that the markers are independent and all have some effect on the analysed trait. However, this is rarely the case. As an example, there are studies indicating that markers located within or near regions rich in genes on a chromosome can explain more variance than markers located between genes. Presently, knowledge of the genetic architecture of complex traits is available, and the amount of information on the DNA markers is continuously increasing in the form of accurately annotated genomes, Quantitative Trait Loci (QTLs) databases, gene expression and gene pathway studies. Models including prior knowledge on different SNPs (single bases in the genome that vary between individuals in the population) have been proposed to improve the predictions in genomic selection and the accuracy of the genomic breeding values.

We have developed and implemented a method for incorporating prior knowledge in genomic selection models by estimating different weights for SNP effects based on their biological function. Starting with simulated data we created different scenarios of the genetic architecture of a trait which were then analysed using the traditional GBLUP model and the alternative model that includes the extra information on the markers. Our results indicate that the alternative model tends to perform better compared to the traditional GBLUP, and yield higher accuracies in most of the scenarios tested. Moreover, we identified two factors that influence how well the alternative model will work: 1. The genetic architecture of the trait – the alternative model performed better than GBLUP when the number of genes controlling the trait was low, but its predictive ability decreased with an increasing number of genes. 2. The external information - we assumed that the location of the genes was known, and defined windows of markers around the genes to have higher weights than the rest of the markers. The results show that larger windows tend to decrease the accuracy because the information provided to the model was vaguer in this case. Including biological information into the model is beneficial as it can increase the accuracy compared to the standard GBLUP. Nonetheless, this benefit depends upon the underlying genetic architecture of the trait and on the quality of the external information.

Apart from sharing our results through a publication we also offer the program CodataGS which we used to implement the analyses (in the free software R) at https://cran.r-project.org/web/packages/CodataGS.

FROM BULL-BASED TO COW-BASED GENOMIC SELECTION

The implementation of genomic prediction was very swift in dairy cattle breeding because tens of thousands of bulls with high accuracy breeding values had their semen stored centrally which facilitated the isolation of DNA genotyping. These bulls with genotypes and breeding values have provided the reference population on which the genomic selection of the next generations has been based. In order to update the reference population with new trait records and remove some of the selection bias that is present in the reference population (bad bulls were never genotyped), genotyping of cows has now become widespread practice.

We evaluated the potential application of a novel approach where the breeding values of the bulls would be based only on the cow data, ignoring the breeding values from ‘old bulls’. Our approach was to test the accuracy and bias of genomic breeding value estimations in simulated population under no, moderate and intensive selection. Accuracy of predicted breeding values for young animals in last generation were compared by applying three different methods: Breed value estimation based on the pedigrees (pedigree based BLUP), Breeding values estimation combining the pedigree and genomic data from genotyped animals (single-step genomic BLUP: ssGBLUP) and so-called SNP-BLUP. In SNP-BLUP the classical pedigree information is replaced with genomic information, so it assumed to be more accurate. In case of no selection in population, the predicted breeding values for young animals were unbiased with high accuracy. In populations under moderate selection, bias was slightly increased and accuracy of predicting young animals’ performance was decreased compared to the population without selection. In populations under intensive selection, estimates of predicted breeding values of young animals were even more biased, compared to populations under moderate selection. The accuracy of breeding value predictions with BLUP was low, whereas ssGBLUP and SNP-BLUP were remarkably higher. This shows that if we move towards cow information and using new methods we can increase the accuracy of our genomic evaluations.

TAKING GENOMIC SELECTION AND SEXED SEMEN TO THE FARM LEVEL

While genomic selection has been mainly implemented to more effectively select breeding bulls, the genotyping costs are now at a level where we can consider genomic selection even at the farm level. We have studied what benefits genomic prediction, in combination with sexed semen and crossbreeding can deliver at the farm. We studied the potential gain using simulation and the practicalities using real data. The simulation of combining crossbreeding, genomic prediction and sexed semen show that genomic selection, sexed semen, crossbreeding (and beef semen) generally improves total herd profit both individually and combined. The next step is to investigate the farmers’ willingness to take up one or more of these alternatives in their dairy farms. In this work we have cooperated closely with an industry PhD student from Viking Genetics (part funded by the Swedish livsmedelsstrategi) who works on a project, ‘Tinder for cows’ in which apps are developed that enable the farmer to identify the best ‘match’ for each cow based on her DNA profile and that of the available breeding bulls.

IMPROVED GENOMIC SELECTION FOR CROSSBREEDING IN PIGS

In a standard pig breeding program, selection is performed in purebred lines with the goal to improve crossbred performance. This structure has some limitations as the pure lines are usually kept in a more favourable environment than their crossbred relatives. Moreover, there are traits of high interest for the crossbred population that are difficult to record in a nucleus. When genomic information is used there is no need for information about the breed, which would make it possible to use information from herds where the sows do not have exact identity numbers. We have developed a novel selection strategy using genomic data in crossbred sows to improve maternal and welfare traits and created a simulation program based on real genomic data from two purebred populations. This approach allows us to capture in the best way possible the way in which different markers are inherited together (linkage disequilibrium) that depends on the structure of each population and speed up the simulation process avoiding unnecessary assumptions. Several scenarios have been tested based on the source of information for the genomic evaluation (i.e. data from purebred lines only, data from crossbred sows only, combination of sources, etc.). The selection strategies are evaluated based on the accuracy of the prediction and the magnitude of genetic gain. The simulation shows that if the breeding goal is a genetic gain of the cross breed the improvement is not as large. This because the gene x environment interaction between breeding and commercial herds. Conversely you cannot expect high genetic gain in commercial herds if the breeding is based only on purebred animals in breeding herds. (SLF funded project as in-kind co-finance).

PROTEOMIC MARKERS OF BULL FERTILITY

While genomics can to some extent predict the phenotype of an organism, large scale measurements of proteins – proteomics – provide a more accurate view of the true phenotype and are easier to interpret. Proteins make up the machinery of the cells, and they mediate signalling and chemical events by catalysing a vast array of chemical reactions. The levels of specific proteins can be used to predict the features that will occur in different crosses in breeding programmes, and they can also be used as an alternative or complement to genomic markers. Seminal plasma (SP) proteins have previously been identified in different species (pig, sheep, and horse). In those species investigations have been performed to relate protein patterns to fertility and also to sperm "freezability". This opens perspectives for using such information in the selection of ejaculates or individuals.

In cattle, male fertility is usually determined from the results of artificial inseminations (AI), estimated from Non Return Rates to oestrus (proportions of cows not coming back in heat after insemination). Due to numerous confounding factors and bias related mostly to the type of female inseminated and effects of the environment, high numbers of AIs are necessary to obtain reliable estimates of bull fertility. This is a limit for the breeding companies, as good fertility estimates is obtained from accumulating data over several years, by which time the bull is no longer in use for AI. Thus it is not possible to define efficient sperm handling or genetic selection strategies. Today, in the context of genomic selection, the situation is even worse as bulls are used at a young age and for a short period. Consequently, very few AIs are performed for each individual. This results in fertility estimates with a very poor value, especially in view of genetic selection.

In the past, differences in fertility between males have been attributed most often to variations in sperm quality. Today, the predictive value of conventional sperm quality traits is usually considered to be low. When our work was initiated, sperm proteomics and especially the contribution of SP was rarely considered. Differences in fertility among bulls with apparently similar sperm quality have been related to variations in SP content and its effects on both spermatozoa and the female genital tract. Sets of proteins from SP have been associated with sperm motility, resilience to freezing and survival in the genital tract in the sheep.

Close to 600 proteins have previously been identified in SP samples. So far, few of the proteins identified were found to be related to fertility. However, most of the analysed samples came from commercial samples diluted with extenders, which leads to lack of identification of about 25 per cent of the proteins. The number of studied animals as well as the number of replicates from each individual were few.

In total, we identified approximately 1000 proteins in SP samples. Out of these, 38 proteins differ significantly in abundance between low and high fertility bulls. In addition, for a set of 18 proteins, significant correlations between their content in SP and fertility indices were found. Among those, 14 correlated negatively with fertility and 4 correlated positively with fertility. From this set of 18 proteins, 4 were previously reported to be related to sperm quality, motility and/or freezing ability in sheep by other researchers. An interesting example is Ubiquitin carboxyl-terminal hydrolase isozyme L3, which increases in abundance with fertility. It has also previously been shown to be involved in the differentiation of spermatocytes into spermatids and to correlate with sperm count, motility and fertilization in humans. To our knowledge, the remaining 14 proteins have not been associated to sperm quality or fertility in the published literature; thus they are new candidates to be screened for bull fertility.

We identified 18 proteins in seminal plasma that correlate with fertility.

We also found that the proteomic profiles of individual bulls are subject to seasonal variations and to differences between technical replicates resulting from analyses performed in different batches. This makes it difficult to interpret the results. However, we managed to identify proteins that correlate with fertility, with less season/batch variation. These results show that the content of some protein/peptide candidates in SP correlates with field fertility. This is a new way to refine bull fertility phenotype. The interest of using these results in the perspective of genomic selection is under discussion between the partners of the project.

In addition, we looked at endometrial epithelial cells from the uteruses of cows to identify possible genes specifically affected by SP, which could explain differences in fertility. When measuring gene expression in endometrial epithelial cells exposed to SP from bulls with high and low fertility (and controls) we found a strong interaction between bull and the cows from which the cell culture originated. For a given cow culture, only a few genes were affected by SP for both bulls, whereas for another cow several hundred genes were differentially expressed with a differential response between the two bulls.

LOST PARTS OF CHROMOSOME IN DAIRY COWS IDENTIFIED

There is a mutation that results in a missing bit of chromosome number 23 in individuals of the SRB dairy breed. The mutation is a so called deletion, which means that a larger chunk of the chromosome (about 525,000 bases in this case) is missing. This specific deletion can result in stillborn calves.

We discovered the deletion as we studied the genomes of cattle of the Holstein, Jersey and SRB breeds. We found 8,480 deletions in 175 cattle, of which most (82 per cent) are not in registered in the databases of previously discovered deletions. In the various missing chromosome pieces there are several inheritance sites that are important for animal health and fertility. In contrast, there are few genes that are related with high milk production in these deletions.

The knowledge is intended to be used in the breeding of dairy cows, and we have created a "deletion catalogue" that will make it easier to find out if an animal has a certain deletion or not, and what it means for the animal. The study also revealed that the RGB breed has a greater genetic diversity, compared to the Holstein and Jersey breeds.

CAN GENE EDITING BRING HEALTHIER LIVESTOCK?

How can genome edited animals be integrated in breeding programs? Disease resistance has been mentioned as one of the main traits of interest for genome editing, and pigs have specifically been discussed in this context. Therefore, resistance breeding in pigs seemed to be a relevant example to discuss. We choose to focus on the two diseases Porcine Reproductive and Respiratory Syndrome (PRRS) and the African Swine Fever (ASF).

PRRS is a disease present in pig herds in many countries around the world. It has been present in Sweden but could be eradicated. This disease comes in waves and leads to a general decrease of reproductive and productive performance in affected herds. There are options for vaccination, but success varies strongly between herds and virus strands. In addition, a gene test to identify resistant animals is offered on the market, but it is unclear how well this test works, and to what extent it is applied. Two different research groups have developed a genome editing approach to target one of the two receptors for the virus, CD163 and CD169, with pigs modified at the CD163 receptor showing some level of resistance to PRRS.

Disease resistance in pigs has been mentioned as one of the main traits of interest for genome editing.

ASF on the other side is a lethal disease, leading to the death of pigs within a few days. The virus was first discovered in Africa. Today it causes major problems in many pork-producing countries in Asia. It can also be found in South and Eastern Europe from where it can spread to Western and Northern Europe. As with the classical swine fever, an outbreak is estimated to costs billions of Euros. Outbreaks in China have already had significant impact on the pork industry. There is currently no vaccine against ASF available. Methods aiming to stop the spread of the disease include early culling as well as surrounding herds and a close surveillance of wild pig populations. However, it has been observed that African wild hogs are resistant to ASF. One option would therefore be backcrossing to integrate the resistance trait into other pig populations. Unfortunately, crossing schemes between commercial pigs and the African wild hog have been unsuccessful. However, especially in Africa, there might be pigs with some degree of resistance to ASF. A number of receptors for the ASF virus have been identified, one of which was further investigated in resistant and susceptible pigs by a research group in Scotland. A mutation was also identified, which has the potential to give rise to resistance.

For both diseases, PRRS and ASF, new strategies for improved resistance of commercial pig herds, including the use of genome editing, have been suggested. It is yet unclear how successful genome editing can be for this purpose. Nevertheless, both diseases display interesting cases for the simulation of a pig population. For the simulation of the breeding programme we suggested three alternatives: 1. susceptible populations as they are, 2. introgression/backcrossing with a resistant population and, 3. the use of gene editing to introduce the phenotype within a short period. In all options we used multiple generations of pure breeding before crossbreeding between a paternal and maternal line. For scenarios 2 and 3 different numbers of individuals were considered during the first generation. We then compared the levels of inbreeding and phenotypic differences.

Inbreeding increased drastically when we used small population sizes in scenario 3. This is important since it is unclear how many animals can be used for genome editing in an economically efficient breeding programme. Furthermore, since pig breeding separates male lines (selection for production traits) and female lines (selection for reproduction traits), more animals would be required for the modification.

We could see that the inbreeding levels became very high already after 50 generations, when the population size was small (for example 15 boars and 30 sows) in scenario 3 using gene editing. We could on the other hand observe that the inbreeding levels were also high in scenario 2 using backcrosses when having a very small population with 10 boars and 10 sows in the initial population. These results show the need to closely observe inbreeding in the population, especially when designing a new population such as in scenario 3. The use of gene editing would probably lead to the use of a relatively small number of founder animals (gene edited animals in generation one) due to costs, but it might be necessary to introduce new gene edited animals after some generations to decrease inbreeding again. On the other hand, both scenarios with backcrossing and gene editing suggest a similar level of improvement of the phenotypes. This suggests that both options to introduce resistance in the population will allow similar results and especially an improvement towards the original population. However, until now, we could only simulate the scenarios with models containing representations of a few chromosomes. A simulation of the entire pig genome will require further methodological development.

Multiple ethical issues need to be considered in connection with these uses of gene editing. One important problem concerns the use of genome edited individuals across breeding populations, including organic farming. Since organic farming has a no-GMO-policy the issue is how genome editing is classified, and where the core argument against GMO lies. If it follows the traditional line between transgenesis and mutation, then genome editing might be regarded acceptable. Including organic breeds in the work to increase resistance could be relevant not only to the individual pigs, but also to limit risks of inbreeding. Including resistant animals in conventional but not organic breeding scheme could both be perceived as unfairly letting organic farmers lag behind, or as saving a genetic resource for the future. The costs of using genome edited livestock in the breeding scheme is highly relevant, in particular to smaller breeding organizations. On the other hand, traditional breeding takes time. Even if it is successful on a national level, it might not have effects on all farms. Farmers who cannot afford resistant pigs from expensive breeding programs will be in need of a "quick fix" if the disease breaks out. Larger farms would expectedly be less vulnerable. Also, diseases evolve and modify, and resistance breeding might not keep pace with the evolution of the disease.

Another important question is how the introduction of resistant individuals will affect the conservation of endangered pig populations. Further simulations of disease spread might be required, and it has to be investigated whether resistant pigs will actually spread the diseases or not. Hence, there will be a need for a further discussions on welfare and integrity, both for the individuals used in various stages in the breeding programs, and for the 'end-products', the animals that will be available to farmers.

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Domesticating a potential oil and cover crop

Domestication of field cress

The world's supply of food depends on a surprisingly small number of agricultural plants. Most of the crops grown today were domesticated several thousand years ago; wheat about 10,000 years ago in the Middle East, maize 9,000 to 7,000 years ago in Mesoamerica, and rice about 8,000 years ago in East Asia.

The plant breeder's toolbox has become increasingly varied and powerful in the last century, with techniques such as hybridization, mutation breeding, genomic selection, and genetic engineering. Recent domestications, however, are rare. The domestication of penny cress (*Thlaspi arvense*) is a case in point, and Salix (willow species) is another. In this light, the ongoing efforts to domesticate field cress (*Lepidium campestre*) are extraordinary.

Still, researchers have been entertaining ideas of domesticating wild plants into oilseed crops at least since the 1950s. A 1966 article reports trials of bitter-cress (*Barbarea vulgaris*) by the Swedish researcher Lars-Åke Appelqvist. In their article 'A search for high erucic acid containing oils in the Cruciferae' from 1965, Goering, Eslick and Brelsford report that 'there has been much interest in the possibility of finding new crops containing industrial oils' and field cress is one of the plants tested for oil composition. Otherwise, when field cress occurs in the scientific literature, it is usually regarded as a weed.

The initiative to domesticate field cress in Sweden came from the late professor Arnulf Merker. The project was first mentioned in the media in August 1993, where Merker was interviewed. Systematic studies of field cress were carried out at SLU in the late 1990s and early 2000s funded by SLU, the Swedish Foundation for Agricultural Research (SLF), and the Swedish foundation for Oilseed Research (Svensk Raps).

Arnulf Merker passed away in 2010. The project nevertheless had enough momentum to carry on, and a strategic grant from the vice chancellor of SLU was secured. The project really took off when Mistra Biotech started in 2012. With the Mistra Biotech program now ending, and with the field cress project continuing in other forms, we might look forward to future developments.

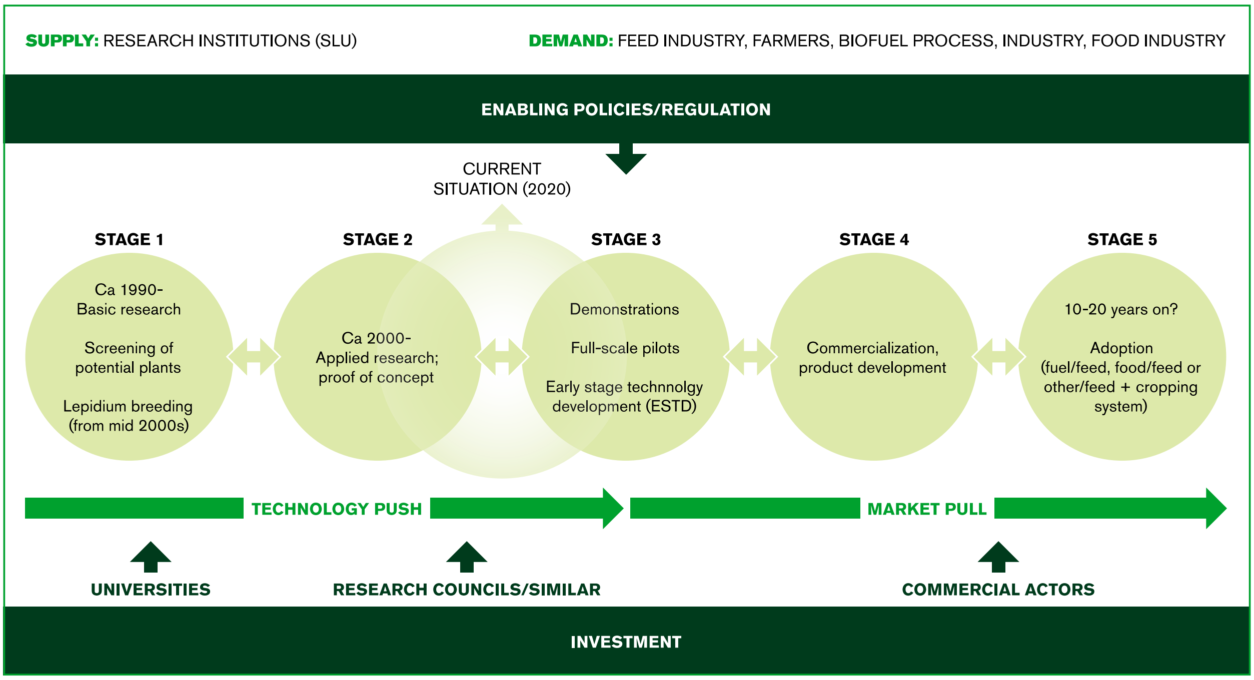
The following vision is a conglomerate of views gleaned from people who have been involved in the field cress research.

It is 2035. Field cress is adopted as an oil and catch crop in Sweden, especially in the north of Sweden. It is undersown in barley, with barley harvested year one and field cress year two. The seed oil is mainly used for biofuel. The rest of the seeds is used as protein-rich feed, mainly locally and regionally. There are plans for introducing field cress in other areas of the world with conditions similar to those in northern Sweden (e.g., parts of North America and Russia).

An alternative vision would be that field cress is established as a food and feed crop. To pursue the different visions, different breeding choices will have to be made. Oil quality would be the main issue along with the seedcake quality if the 'food and feed' track were chosen.

The project might soon be facing a difficult transition from research and invention to adoption and use. It is common to visualize the process in which a product or a new crop is invented, developed and finally adopted as an innovation chain including five stages. The first two stages in the chain involve basic and applied research. The third stage involves early-stage technology devel-

The initiative to domesticate the wild plant species field cress into a new oil and catch crop came from Arnulf Merker (1945-2010), Professor at SLU, around 30 years ago. He had a deep interest in biology and nature, and he was strongly engaged in how to make agriculture more environmentally friendly. He was also deeply involved in international projects for improving plant breeding programmes and seed businesses in Africa, Central America, and Asia. As an expert on rye and wheat breeding, he contributed to the establishment of triticale as a new crop in Sweden. Improvement in wheat, rye, and barley through crossings with related wild species was the main focus in his research, and he worked with a large number of traits in these crops. Arnulf saw the potential of using modern breeding techniques to breed for new and better crops, and he defended the use of these techniques in the early GMO debates in media. In addition to his research, Arnulf had a strong social commitment and a dedication in politics. In the 1980s he represented the Left Party at the municipal level. The photo comes from an article in Helsingborgs Dagblad in 2007, where Arnulf is seen in one of his trials with field cress. Now, thirteen years later, his idea has come close to realisation. (Photo: Anna Bank).



The process in which a new crop is invented, developed, and finally adopted.

opment including full-scale pilots and demonstration. The fourth and fifth stages involve commercialization and industry adoption. It seems fair to say that the field cress project is now in between stages two and three. Funding for basic and applied research typically comes from public sources, as has been the case with field cress. Since the project is still not ready for commercialization, it is difficult to find commercial actors to invest in the further development. It is thus crucial to continue to search for some public funds for completing the stage two and three studies after Mistra Biotech completion.

In addition, there is uncertainty regarding the regulation of gene editing in the EU. If the current restrictive regulation were to be changed, field cress breeders would have more tools available.

Breeding the new crop

Field cress has the potential to become a crop with excellent properties. It is biennial and can function as a catch or cover crop. It covers the field during the winter when the arable land would otherwise be bare and "catches" the nutrient fertilizers from the field. Field cress can be cultivated together with barley or wheat.

Oil from the seeds can be used to replace the fossil oil used for instance in the chemical industry, and to get healthier oils for human consumption. There is currently no oil crop that tolerates the cold winters in the north of Sweden, but after being completely domesticated, field cress could be cultivated throughout Sweden, and other parts of the world as well.

Field cress has a couple of ”wild” traits that are not fit for cropping, but luckily we have identified a genetic variation in these traits, which means that the wild traits can be improved by breeding. These variations were found both within the field cress species and in other species within the genus *Lepidium*, with which interbreeding is possible. The identification of this variation in the genes also provides information on how the species within the genus *Lepidium* have changed during the evolution – field cress is more closely related to Smith's pepperwort (*Lepidium heterophyllum*) than to the pepperwort *Lepidium hirtum*. Field cress is also fairly closely related to the model plant thale cress (*Arabidopsis thaliana*), a species that is very well studied in terms of genes that control important traits. This is important knowledge that we have made use of. We have looked for genes in field cress that are similar to known genes in thale cress, and thereby we could identify genes regulating vernalisation, florescence, pod shattering, and the quantity and quality of oil.

We have improved a number of major traits, and importantly we have managed to stack these improved traits into single field cress breeding lines, which combine high seed yield, pod shatter resistance, synchronous maturity, and increased oil content. These achievements have been obtained with conventional plant breeding methods, including the collection, characterization and evaluation of plants, and breeding of selected plant lines over a number of generations through selfing, hybridization and backcrossing. We have also employed modern breeding tools such as genetic modification and gene editing. Very recently, we have managed to develop an efficient CRISPR/Cas9 protocol in field cress paving a novel way for future breeding using this technique.

The chances to improve a crop though conventional breeding increase with a larger number of plants carrying different varieties of genes. Therefore, we based our work on a large and diverse collection of plants; 26 accessions of field cress and 15 accessions of other *Lepidium* species, which we have obtained from various gene banks and botanical gardens in Europe and USA, as well as 36 accessions of field cress populations collected across Sweden.

OIL CONTENT AND QUALITY

In its natural populations, field cress has less than 20 per cent seed oil. The oil quality in the wild type is not suitable for foodstuff due to its high content of erucic acid. The level of seed oil has to be at least around 30 per cent for field cress to be economically viable. As a comparison, winter oilseed rape, the major oilseed crop in Sweden, contains more than 40 per cent seed oil. However, due to its weak winter hardiness it is only cultivated in southern Sweden. During the breeding-process we have made continuous chemical analyses of oil quality. We have also analysed some other major components such as cell wall polysaccharides (see page 59).

INCREASING THE OIL CONTENT

We aimed at increasing the oil content to over 30 per cent by crossing the available accessions of field cress and other *Lepidium* species. The oil content in the wild field cress that we have analysed varies between 12 and 18 per cent. The other *Lepidium* species show similar oil contents, except *L. graminifolium*, which has about 38 per cent. We have made a large number of crossings between *L. graminifolium* and field cress. Hybrids between species often have lowered fitness, and unfortunately the embryos of the hybrid seeds failed to fully develop. The hybrids between field cress and other *Lepidium* species were more viable but they did not have an increased oil content.

Sticking to only field cress, we selfed and/or crossbred different genotypes with above-average oil content, and their progenies with increased oil content were further bred by selfing and crossing for several generations. In the end, the oil content of some lines were increased to 27-29 per cent under greenhouse conditions. In the field, the best of these lines produced 27 per cent oil in their seeds. Our efforts indicate that a further increase in oil content based on crossbreeding is difficult to achieve.

Can the oil content be increased in other ways? In parallel with conventional breeding we have used genetic modification. Seed oil content is controlled by a large number of genes, which are associated with multiple steps of oil biosynthetic pathways. Modulation of single genes usually results in at most moderate increase in seed oil content. Therefore, we aimed

The goal is to use the field cress seeds as a source of oil for biofuel or for consumption. The rest of the seeds, the seed cake, can be used as protein-rich feed. (Photo: Cecilia Gustafsson) at regulating transcription factors – genes that can regulate multiple steps simultaneously. In the model species thale cress, a key transcription factor has previously been identified to be involved in the regulation of oil accumulation. Moreover, hemoglobin genes from both thale cress and sugar beet (*Beta vulgaris*) have also previously been shown to increase the oil content when overexpressed in thale cress. We introduced these genes into field cress and the oil content was indeed increased up to 30 per cent. The composition of the oils in the different genetically modified lines did not differ from that of the wild type plants. This is the first piece of work showing that genetic modification can be used in this wild species to speed up domestication.

HEALTHIER OIL

Unfortunately, the fatty acid composition of the oil of the wild type of field cress is not suitable for food use. This is mainly because of high levels of erucic acid, a long chain fatty acid. Erucic acid is the second most abundant fatty acid in the seed oil with levels between 23 and 28 per cent. Erucic acid at high levels is very unhealthy and makes the oil inedible. EU regulations have a limit of at most five per cent erucic acid in food oils. Another problem with the seed oil of field cress is its high levels of linolenic acid, a polyunsaturated fatty acid. It dominates the seed oil with levels between 40 and 46 per cent and is prone to oxidation, especially at elevated temperatures. This gives field cress oil a short shelf life, and limits its use in food processing.

We wanted to develop a field cress oil that is predominantly rich in oleic acid and devoid of erucic acid, for food or industrial applications. By decreasing the expression of two genes, encoding enzymes that regulate the fatty acid composition, the level of the healthy fatty acid (oleic acid) was increased from 11 per cent in the wild type to over 80 per cent in the seed oil from the genetically modified field cress lines. At the same time, erucic acid was reduced from 20 per cent down to 0.1 per cent and the linolenic acid level was decreased from 40 per cent to 2.6 per cent. The high oleic acid trait has been kept stable for three generations of the genetically modified field cress. This shows the possibility to apply genetic engineering on field cress to tailor-make oils for various end uses.

PRODUCTION OF WAX ESTERS

Wax esters have high resistance to hydrolysis, making them excellently suited as lubricating oils. Seed oils do not normally contain wax esters, with one exception, jojoba (*Simmondsia chinensis* L.), a perennial desert shrub. However, this species is not suitable for large-scale cultivation. It is possible to produce wax esters synthetically, out of fossil oil, but this alternative is expensive and results in poorer oil quality apart from more negative impact on the environment. Breeding plants to make them produce wax esters would be a sustainable alternative.

We tested whether wax esters of different molecule sizes could be produced in field cress by introducing three genes from jojoba into field cress. The experiment was successful. Two of the genes gave rise to relatively short chain wax esters in the seed oil of field cress, while additional expression of the third gene resulted in an increased level of longer chain wax esters. The results indicate that field cress has the potential to be developed into a new industrial oil crop for wax ester production.

REDUCING THE CONTENT OF GLUCOSINOLATES IN SEEDS USING GENE EDITING

Glucosinolates are secondary metabolites in plants. They can be toxic in certain forms and levels. Glucosinolates are chemically stable before coming into contact with the enzyme myrosinase, which is also present in plants. Such contact occurs when the plant is damaged, for example by an herbivore. The reaction results in a pungent smell, also called a mustard bomb, which protects the plant from herbivores and pests. Glucosinolates are common in species of the order Brassicales, including field cress. Since they are unhealthy at high levels, the glucosinolates limit the use of the field cress seed cake or meal that remains after oil extraction, which would otherwise be useful as feed or food purposes. Most plants contain a mix of several different glucosinolates. Like yellow mustard, field cress contains a significant amount of sinalbin, which is the mildest glucosinolate in terms of flavour and toxicity, whereas it only contains trace amounts of other glucosinolates. In the model plant thale cress, knocking out two glucosinolate transporter genes has been shown to block the accumulation of glucosinolates in the seeds. Using gene editing, we aim to knock out the corresponding two genes in field cress. We have obtained gene edited lines of field cress with mutations in the genes individually or in combination, in order to determine the effects of the genes individually and in combination. Some of these lines are currently under vernalisation for flowering, and we look forward to evaluating the glucosinolate contents in the seeds of these lines.

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AGRONOMIC TRAITS

Since 2015, we have conducted field trials with field cress at different locations in Sweden: Lönnstorp (in Skåne), Umeå, Lännäs (in Ångermanland), and Lanna (outside Lidköping). The first field trials took place in Lönnstorp with 176 lines that were the results of breeding efforts since 2012. Those lines were selected based on traits such as seed yield, pod shatter resistance, oil content, and synchronous maturity.

SEED YIELD, EARLINESS AND RELATED TRAITS

The seed yield per plot in 2015 was as high as 4.5 t/ha. From the 176 lines we selected 44 lines that were undersown in barley in the following year. Additionally, based on overall evaluation of the performance (such as seed germination, plant vigour, weed competitiveness and earliness), 24 lines (eight early maturing and 16 late maturing) were selected for multi-environment field trials at all four trial sites listed above. At Lanna, the average seed yield for the early maturing lines varied from 2.5 to 3.6 t/ha, while the average for the late maturing types was lower, up to only 2.6 t/ha.

We continued our studies of the 24 lines at Lönnstorp and Umeå in the spring 2017, in order to compare mono-cropping versus undersowing. Comparing seed yield data from mono-cropping during 2016/2017 and undersowing in 2017/2018 showed that undersowing is a preferable approach for field cress. It resulted in a higher average seed yield, mainly because undersown field cress competes better with weeds after the barley has been harvested. Later, 11 of the 24 lines were selected and field trials were conducted at the Lönnstorp research station on larger plots (200 m^2 to 400 m^2) for further evaluation and selection. Based on the seed yield data from these and previous trials, the top five high yielding breeding lines were selected for large-scale field trials during 2019/2020.

In parallel to the field trials, a large number of new field cress hybrids were generated in 2015 by crossing different breeding lines. These hybrids were grouped into families, and about 30 families were selected for breeding during 2016. The main objective of this cross-breeding is to further improve the desirable traits. One hundred offspring breeding lines, derived from these families, were planted at the Lönnstorp research station. Based on the data from 2018, we selected individual plants and breeding lines for field trials 2018/2019.

Regarding earliness, some of our lines mature three weeks earlier than the late types. This makes them more suitable for cultivation in the north of Sweden. Our research showed that pod density, pod sterility and inflorescence length are major traits contributing to seed yield.

The top performing lines from the trials in 2018/2019 are so to say the final outputs of Mistra Biotech's contribution to the domestication of field cress. The highest seed yields were 4.7, 3.9, and 2.7 t/ha at Lönnstorp, Lanna and Umeå, respectively. It should be noted, however, that due to unoptimized agronomic practices that have affected the overall performance of the selected lines, the field trials might not show the full potential of the improved field cress lines.

In the selection breeding parental plants have been chosen based on traits such as seed yield, pod shatter resistance, oil content, and synchronous maturity. (Photo: Mulatu Geleta Dida)

In the wild, pod shatter is vital for the plant to be able to spread its seeds, but in the agricultural field it can result in yield losses of up to 50 per cent.

PERENNIALITY

There are several potential benefits of perennial crops. Because of their extended growing season and deep roots, they may be more drought tolerant, require less fertilizers, and prevent water runoff. They can also reduce tillage. Field cress is biannual – it grows a rosette in year one and sets flowers and seeds can be harvested in year two. However, we have found that some varieties have ratooning ability and can be harvested more than once under greenhouse conditions. Because *L. heterophyllum* is a facultative short-lived perennial species, closely related to, and easily crossable with *L. campestre*, we generated *L. campestre* × *L. heterophyllum* hybrids from various parent plants and have evaluated the offspring since 2012. Some of the offspring became strongly perennial, and produced satisfactory seed yield up to four times (ca. 3 t/ha in average) but with a bit to high levels of pod shattering. Similarly, some plants showed both perenniality and pod shattering resistance, being more perennial than any of the parent plants. We have also selected *L. campestre* × *L. heterophyllum* hybrids and crossed them with *L. hirtum* in an attempt to further improve perenniality and other desirable traits. It remains to see if the offspring will be more productive and perennial. We are convinced that with appropriate selections of parent plants from the species involved in a crossbreeding, a high yielding and winter-hardy domesticated perennial *Lepidium* can be obtained within a couple of decades, particularly if breeding is guided by deep understanding of plant evolution and domestication as well as by genomics. Additionally to perenniality we have also identified genotypes with favourable root systems.

POD SHATTERING AND SEED GERMINATION

Wild field cress has a trait that is most unfavourable in agriculture, namely pod shatter, which means that seeds fall to the ground before harvest. In the wild, pod shatter is vital for the plant to be able to spread its seeds, but in the agricultural field it can result in yield losses of up to 50 per cent. We screened our collections to identify genotypes that have a relatively low pod shattering. This was done by exposing fully mature plants to heavy rain, wind and storm for an extended period in the field. Then we crossed promising plants, followed by cycles of selection, backcrossing and selfing for a number of generations. *L. campestre* × *L. heterophyllum* interspecific hybrids were major contributors to the development of pod shatter resistant lines, while the interspecific hybridizations attempted between *L. campestre* and *L. graminifolium* as well as between *L. campestre* and *L. draba*, were unsuccessful in this respect. Overall, reducing pod shattering in *Lepidium* was highly successful and one of the major achievements of this project.

Taking a closer look at this trait, and the genetics behind it, we found another direct way to reduce pod shattering. During seed maturation, three distinct tissue elements of the seed capsule are believed to contribute to the rupture of the seed capsule: a separation layer, cells with a secondary wall adjacent to the separation layer, and a valve endocarp layer with a secondary wall. Some genes that specify the valve margin have previously been identified in the model plant thale cress. One of these genes plays a crucial role in both lignified and separation layer development. We decided to find the corresponding gene in field cress and reduce its expression level, using genetic modification. The majority of the genetically modified field cress lines had a clearly increased resistance to pod shatter by reducing the expression level of the above-mentioned key gene, as compared to the wild type, and this resistance has been maintained in four following generations. Our analyses show that it is possible to generate field cress lines with a desirable pod shatter resistance by down regulating a single gene, thus speeding up the domestication process. We have also (in collaboration with the John Innes Centre, UK), modified field cress with a gene from the plant species *Brassica rapa*, which also resulted in an increased pod shatter resistance. However, this resistance was clearly reduced in the next generation of offspring.

The rate of seed germination varies among field cress populations and breeding lines. Some of the lines that have been bred for other useful traits have low germination rates when sown directly after harvesting. In particular, breeding for pod shatter resistance resulted in strong seed dormancy in some promising lines. In the beginning of 2016, these shatter resistant lines were crossbred with lines that have excellent seed germination records. After several rounds of breeding, lines that combine the two traits were developed, which is a good example of successful trait pyramiding research.

We also stacked the traits of high oleic acid lines and improved pod shatter lines or wax ester lines. Reciprocal crosses were made for both combinations (high oleic acid × improved pod shatter, and high oleic acid lines × wax ester) for up to three generations, resulting in maintained reduction in pod shatter and high content of oleic acid and wax esters, indicating that all these genetic modifications are stable in the hybrid lines.

PATHOGEN AND PEST SUSCEPTIBILITY

A crucial factor for field cress as a potential crop is its resistance or tolerance against pathogens and pests. This is particularly important if the crop is to be grown in organic farming systems (which exclude most chemical pesticides). Field cress can be a host for fungal pathogens such as *Plasmodiophora brassicae* and *Alternaria brassicae*, both of which attack rapeseed. However, the most serious fungal pathogen attacks that we found in field cress were caused by dark spot (*Alternaria brassicicola*) and gray mold (*Botrytis cinerea*). They were also observed in the wild field cress populations in Sweden during our collection in 2012. Luckily, we have also identified field cress genotypes that are resistant to these two pathogens under field conditions.

Breeding achievements in some important traits in field cress during the eight years of Mistra Biotech using methods including genetic modification (GM) or conventional methods (non-GM).

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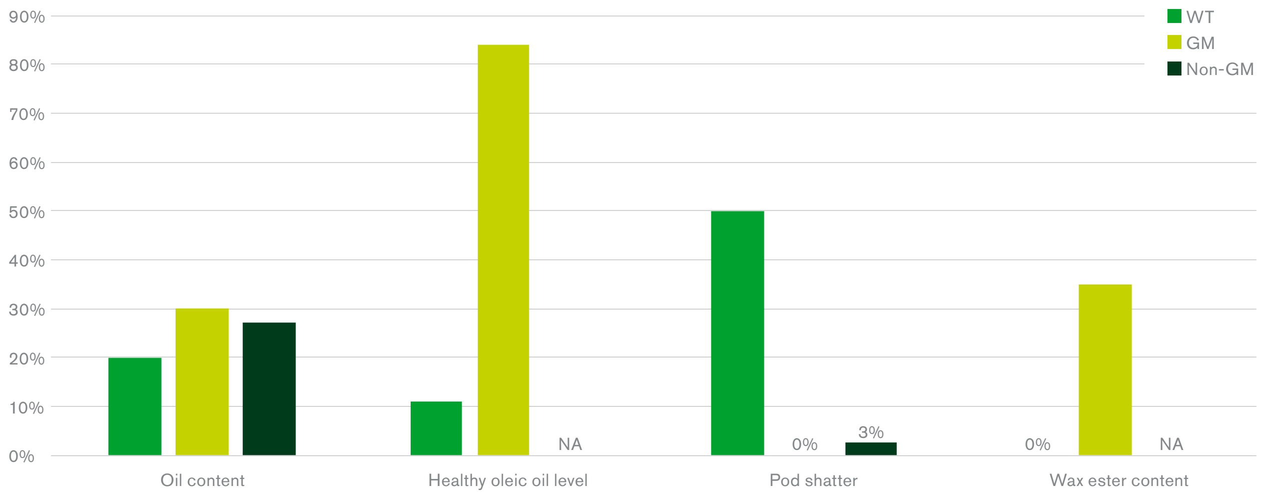


Improvement in some of the field cress traits. Wild type, altered through genetic modification and through cross-breeding.

Oil from the seeds can be used to replace fossil oil used for instance in the chemical industry, and to obtain healthier oils for human consumption.

Additional quality analysis of seed components

We have already mentioned the high oil content, the tailored composition of fatty acids, and the lowered glucosinolate levels as crucial for the commercial viability of field cress. In addition the seeds of field cress contain around 20 per cent protein, 40 per cent dietary fibre and seven per cent ash. The dietary fibre composition determines the nutritional quality of the seeds, for example fermentability and pre-biotic properties. We have characterized the compounds in the seeds from our different field cress breeding lines. The oil composition differs between seed samples depending on their geographical origins. Sterols are present in plant oils, and some of these are known to reduce human blood cholesterol levels. As an example, the levels of some plant sterols were higher in the seeds from Sweden, while the level of other sterols were lower, compared to samples with American origin. These geographical differences indicate a genetic variability that may be useful in future plant breeding.

TOCOPHEROL, TOCOTRIENOL, AND STEROLS

Tocopherols and tocotrienols are antioxidants that protect the oil from oxidation in the seeds. They possess vitamin E activity and are thus beneficial for humans. The amount of these compounds varies substantially between different plant oils. In field cress we only detected γ-tocopherol. The levels varied between 1.3 and 2.5 mg/g. This is about two times higher than what is generally found in canola oil.

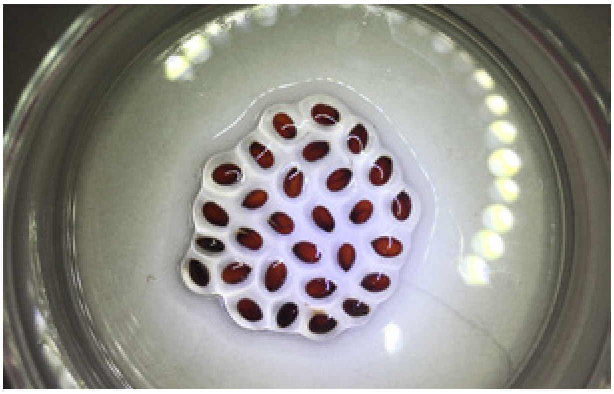
Among the sterols present in field cress seed oil we found that γ-sitosterol dominated with an average content of 2.7 mg/g, followed by campesterol (2.0 mg/g), brassicasterol (0.8 mg/g), cholesterol (0.7 mg/g), avenasterol (0.5 mg/g) and stigmasterol (0.4 mg/g). This resembles the sterol composition found in canola oil. The high level of cholesterol found in field cress is notable and very rare in the plant kingdom.

DIETARY FIBRE COMPOSITION

We determined the dietary fibre content and composition of ten randomly selected samples of field cress seeds. The average content was 36 per cent in dried seeds. We found high levels of lignin, which probably originates from the strong and protective seed coat covering the field cress seeds. We also found low levels of fucose residues. Residues of this sugar is a marker for the presence of xyloglucan, a hemicellulose that is present in the primary cell wall of all vascular plants.

FIELD CRESS AS A SOURCE OF PECTIN?
Pectin is a polysaccharide used in food as a gelling agent and stabilizer, and as a source of dietary fibre. When field cress seeds are submerged in water, a layer of transparent gel is formed on the surface of each seed. We found that this layer consists of pure pectin. Part of the pectin is water soluble and thereby possible to isolate by filtration. The remaining gel can be separated from the seeds mechanically and filtered again to a second gel-forming fraction. The pectin solutions are transparent, colourless, and odourless. The first water soluble fraction contained 79 per cent polysaccharides, and the gel-forming fraction from the second step contained 62 per cent (based on dry matter). The two fractions have similar compositions, but we saw small differences in the pectin molecules, which may be an important observation for future applications of the pectin. The fact that it is possible to isolate a fairly pure pectin in a simple way without breaking the seed coats opens for utilisation of this resource as a step before the oil is extracted. It may increase the commercial value of field cress.

PIGS EATING FIELD CRESS CAKE
The potential value of all parts of a crop need to be considered to obtain the best possible economy. A substantial amount of seedcake is produced as a side stream when oil is extracted from the seeds. The seedcake contains energy and valuable nutrients, and can therefore be useful as animal feed. We mixed four, eight and twelve per cent seedcake from field cress into a common cereal-based feed and gave it to growing pigs. Twelve per cent was set as an upper limit since in its wild form the field cress seeds contain relatively high amounts of the unhealthy glucosinolates (see page 54).



When field cress seeds are submerged in water, a layer of pure pectin is formed around the seeds. Pectin is used in food as a gelling agent.

The field cress seedcake also contains relatively high levels of insoluble fibres. This, together with the glucosinolate content, was found to result in a somewhat lower digestibility (how much of the nutrition the pig actually utilizes), but no negative health effects of the new feed were observed. The digestibility of the field cress feed was lower among the young growing pigs than among adults. This may be a reason to give field cress seedcake to adult animals (sows and ruminants) rather than to young animals.

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Developing the tools

To be able to make use of new breeding technologies and methods, and to analyse the outcome, knowledge about the specific organism is needed. We had to develop and adjust the protocols and methods used in the lab, especially tissue culture that is a crucial step in many molecular breeding techniques. For instance, many new breeding technologies are tissue culture-based. Tissue culture methods often need to be optimized for each new plant species. In order to be able to use new breeding tools for improving important traits, it is crucial to develop and adjust tissue culture based protocols and methods to make them work with the species we have in our program. This is the case for field cress. A considerable amount of such technical work was needed to establish an efficient genome editing method.

METHOD DEVELOPMENT FOR GENE EDITING
With the gene editing method CRISPR/Cas9 it is possible to target specific sites in the DNA of an organism and generate point mutations, without leaving any external DNA in the genome. An efficient way to do this is to introduce the CRISPR/Cas9 vector, which finds and cuts the targeted part of the DNA, into protoplasts (cells that had their cell walls removed). A working protocol for protoplast regeneration is a key to get a successful gene editing method. We have tested several protocols developed for other species and studied a large number of key factors that may contribute to the optimization of the gene editing method. Finally, we successfully established a relatively simple and efficient

protocol for protoplast regeneration of field cress with a regeneration frequency that is often close to 100 per cent, without introducing any new DNA. We are now inducing flowering in our first gene edited field cress lines using cold treatment. In addition, more gene edited plants are on their way and will be evaluated for potential mutation once the shoots are big enough.

In parallel, we have used the CRISPR/Cas9 vectors to obtain mutant field cress lines through transformation with *Agrobacterium* (a common method used in genetic modification). With this method the CRISPR/Cas9 complex will be left in the field cress genome after integration. It is however possible to get rid of the CRISPR/Cas9 complex from the genetically modified lines by selfing, which results in gene edited lines without the extra DNA insertion. So far we have obtained a number of field cress lines modified in this way. The screening for more gene edited lines is still ongoing.

Both protocols are now routinely used to deliver CRISPR/Cas9 vectors into field cress. In the future, focus will be on the protoplast approach for modifying new traits in field cress breeding to avoid the work with backcrossing. We have found that, with small modifications, we can use these protocols to establish or improve protoplast regeneration protocols for several other *Brassica* species as well, including the model plant thale cress. This can be important for researchers working with these species in the future, both for basic and applied research.

METHOD DEVELOPMENT FOR DETERMINATION OF SINALBIN

A commonly used method for the determination of glucosinolates includes purification by solid phase extraction and liquid chromatography. This is a rather complicated method that includes several steps, which makes it difficult to get comparable results in the analyses of samples. The method was in need of further development. In a study carried out by one of our master students we found that crude extracts from field cress could be analysed by chromatography without the solid phase extraction step. This dramatically simplified the protocol and improved repeatability. We now have a working method for the analytical procedure, which just needs to be streamlined for a higher throughput of samples.

EXPLORING THE GENOMICS OF FIELD CRESS

In modern plant breeding it is essential to have detailed knowledge about the genome of the plant. In particular, knowledge about the functions of various genes and the genetic variations within the species can be used to speed up the breeding process. Prior to the start of Mistra Biotech, very little was known about the genome of field cress. We have performed research that has filled much of this gap in knowledge.

MAPPING THE GENOME OF FIELD CRESS

Genetic maps are essential for modern breeding, and they are necessary for the identification and cloning of genes that regulate important traits. Our principal goal was to construct a genetic map –for the first time ever in field cress – using integrated approaches of genetic, comparative and cytogenetic map analyses. In total, we have mapped the genomes of 503 F2 interspecific hybrid field cress individuals. Comparative analysis demonstrated that about 57 per cent of the sequenced loci in the field cress genome are congruent with the genome of the model plant thale cress. Our results also suggested a previously unknown set of chromosomes, which predates the ancestry of the plant family Brassicaceae. The molecular and genetic mechanisms underlying the domestication traits of field cress remain largely elusive. Our overarching goal was therefore to identify so-called quantitative trait loci (QTLs), sites in the genome that are fundamental for the domestication. Multiple QTL mapping algorithms were used to detect and estimate QTL effects for seven important domestication traits. We found 27 QTLs with medium to high heritability, implying that genomics-assisted selection could potentially deliver domesticated lines in field cress breeding. The use of high throughput genotyping can accelerate the process of domestication in novel crop species. These were the first ever QTL mapping analyses in the field cress genome, and they may lay a foundation for genomics-assisted breeding in field cress. We also analysed several genomic selection models used for other species, with respect to both the prediction accuracy and the genetic gain from selection in field cress. Selection based on whole-genome predictions with high accuracy can replace selection based on so-called phenotyping (evaluation of the actual plants) or simpler marker-assisted breeding protocols.

USING GENOMIC INFORMATION AS A RESOURCE IN FIELD CRESS BREEDING

As a contribution to the development of genomic tools and resources for field cress, we developed over 1600 microsatellite markers that can help us find differences in the genomes of individual field cress plants. Of these markers, 120 were used for preliminary assessments of genetic diversity and of the relationships between field cress populations. The transferability of these markers

We found variants of 30 genes that affect important traits in field cress, thus it is possible to select the right individuals knowing that this will affect the offspring's traits in the right direction. (Photo: Mulatu Geleta Dida)

to other *Lepidium* species that are used in the breeding process was also analysed. Several markers have been developed that that can be used to identify interesting *Lepidium* hybrids without a need for phenotyping. As collaborative research activities within an SSF-funded project (*Oil Crops for the Future*), we also performed a comparative genomic analysis using *Arabidopsis* and *Brassica* genomic resources. In this analysis, we identified partial sequences of 30 genes coding for major traits that were targeted in the field cress domestication. We sequenced a number of samples from different breeding lines, and analysed the variation in the coding regions of these genes. Those genes determine for example the time of flowering, oil quality, and pod shattering.

Comparisons between the genomes of field cress and the model plant thale cress have shed some light on the evolution of the five thale cress chromosomes from the ancestral species that had as much as eight chromosomes. Based on this relationship, 24 genes that are known to regulate desirable traits such as pod shattering, winter hardiness, flowering time, inflorescence length, oil content and quality in thale cress could be mapped in field cress. We also identified QTLs for stem growth orientation, plant height, number of stems per plant and perenniality.

We have also analysed 864 individual plants representing three populations (288 individuals per population) to find markers for seed dormancy, pod shatter resistance, inflorescence length, pod density and plant height. Both genotypic and phenotypic data are being processed for QTL mapping of the aforementioned traits, and previously unidentified markers have been identified.

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A healthy and sustainable potato

Potato is the most important non-grain crop in the world and the most high-yielding crop in Sweden. Many people all over the world eat potatoes but the starch-rich tubers are also valuable for various industrial purposes. Potato breeders and growers are however constantly at war with a number of diseases and insect pests. In particular late blight, caused by *Phytophthora infestans*, leads to major problems in potato cultivation around the world. At the same time, there are problems with over-fertilisation of our surface waters. We could reduce over-fertilisation if agricultural crops such as potatoes were better at taking up nitrogen from the soil. It is also possible to make the potato's carbohydrates slower, and thereby healthier, by changing the starch composition. The goals of our potato breeding project have been to produce resistance to diseases, increase the nitrogen uptake and raise the amylose content.

Breeding of potato in the Fennoscandinavian region

Sweden, Norway and Finland have the potato in common. We all prefer the floury cultivars, while elsewhere in Europe people prefer the firmer potatoes. We have the cold northern climate in common, with long days in the short summer, and we have problems with the same kind of pathogens. On the other hand, we currently have no major problems with drought, which is why drought tolerance has not been a particularly prioritized potato trait in this region. The three countries should join hands and cooperate to develop new better potato cultivars. A coordination of the breeding activities in the Fennoscandinavian region would be of great benefit to all involved stakeholders.

Having previously decreased for decades, potato consumption has remained steady at just above 40 kg per person and year in Sweden the last 20 years. Do we want to continue to eat potatoes in the future? Can we grow potatoes in more sustainable ways than today? Yes, but sustainability requires smart strategies.

We need potato varieties that do not need to be sprayed with fungicides. They also need to be adapted to a future climate, which will most likely be warmer and wetter in the north. The particular conditions that we have in the north requires an early tuber maturity due to the short summer season. And, in addition to late blight, there are other potato diseases that are common in this region. In addition we will have to deal with new pests that are expected to find their way to this region as the climate changes.

The Fennoscandian market is too small to motivate the profit-driven breeding companies to invest in potato breeding that matches the specific requirements of this region. Consequently we must rely on public investment to develop such new varieties, and such financial support must be maintained for a long time. There are several reasons for public investment in potato breeding in the Fennoscandian region:

- National food self-sufficiency has high priority.
- Environmental concerns, as expressed in the environmental quality objectives ‘A non-toxic environment’ and ‘A varied agricultural landscape’.
- The gross return on investment is generally very high for plant breeding.
- The industry provides employment opportunities.
- The Fennoscandian region is well suited for potato cultivation.
- Existing cultivars need frequent fungicide treatments and are not adapted to the long days in the summer.
- Potato is an essential component in the cuisine and the culture of the Nordic countries.

Once a new potato variety has been developed from genetic material with many different sources, it has to be tested on a larger scale in an efficient way. In that stage, and in the subsequent commercialization, collaboration with an established plant breeding company can be useful. In Norway the plant breeding company Graminor has an agreement with the private partner Agrico, giving Graminor the right to market the cultivars at a national level while Agrico has gained the right to market the cultivars abroad. Graminor is largely funded by the state but it also has private part-ownership.

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Amylose potato

Potatoes are a good source of vitamins, minerals, fibres and even premium quality proteins. However, the carbohydrates in the form of starch are, besides water, the largest component of the potato, which unfortunately leads to a high glycemic index (GI). But what if those carbohydrates could modified to be more slowly digested, or even better, resistant to digestion? Food products containing resistant starch or a low GI starch are beneficial components of the diet. Resistant starch functions as a dietary fibre with benefits for our bodies. As such, it lowers the glucose levels and the insulin responses, increases fecal output and reduces the fecal transit time. It decreases the calorie content in foods, which is related to weight loss, and it promotes the growth of beneficial gut bacteria.

Potato is the main crop grown for starch production in Sweden and northern Europe. The extracted starch is used in many different applications, both in food and non-food products. In addition to its health effects, a high amylose starch has improved properties as a raw material for the production of biobased plastics. Potato starch is usually made up by two components, 25 per cent amylose and 75 per cent amylopectin, of which the amylopectin is responsible for the high GI. By turning off two genes, named the starch branching enzyme genes (*sbe1* and *2*), the structure of the amylopectin can be changed and the content of amylose can be increased. The resulting starch will be a slow or even resistant carb, in contrast to the fast carbs that it replaced.

SILENCING THE BRANCHING GENES

Initially, we developed high amylose potatoes by using traditional gene technology, namely RNAi. We inserted gene fragments in the potato genome that inhibit the potato's own gene expression. The ensuing field trial showed that we had obtained lines that varied in amylopectin structure and amylose ratio. The total starch content was lower in the high amylose lines than in their parental lines. However, there was also an increase in tuber, somewhat compensating for the lower starch yield.

A NEW WAY OF GENE EDITING POTATOES

When the genome editing tool CRISPR/Cas9 became available, we were fast in implementing it in potato. The new method turned out to be highly efficient in inducing mutations and knockout genes, without even inserting any new DNA in the genome. Instead of allowing the DNA, coding for the scissor, to code for the RNA and protein that make up the CRISPR/Cas9-complex inside the plant, the RNA and the protein complex are produced outside the plant before the protein complex is introduced into the cell where it does its job. We took cells from the potato, removed the cell walls and added the CRISPR/Cas9 complex (i.e. the gene scissor and not the DNA that encodes for the scissor). Then the cell cultures were grown into plants. We then checked that the mutations were in the right places.

In this way, we could develop a second-generation non-transgenic amylose potato with the same two genes, *sbe1* and *sbe2*, mutated both individually and in combination. Since potato is tetraploid (it has four sets of chromosomes), a double gene knockout would need mutations in all eight gene copies (alleles). However, since we did not know if a complete knockout of both *sbe1* and *sbe2* would affect plant growth negatively, or perhaps even be lethal to the plant, we also studied lines with minor *sbe2* activity. Based on their differences in number of alleles mutated, the regenerated lines were divided into three groups; 1. All alleles of *sbe1* mutated, 2. All alleles of *sbe1* as well as two to three alleles of *sbe2* mutated, and 3. All alleles of both genes mutated. Greenhouse trials with thirteen lines representing the three groups revealed that we had developed lines that varied in terms of amylose ratio and amylopectin structure. Remarkably, with this modern plant breeding technique, we were able to develop potatoes with mutations in all eight alleles, leading to a pure amylose starch. This had not been possible with traditional gene technologies like RNAi.

The high amylose traits had an expected negative effect on tuber yield, dry matter and tuber phenotype, which was also seen in the amylose potatoes developed with RNAi. Further application studies of the developed starches are needed to investigate possible positive effects both for our health and for the environment.

FUNCTIONAL PROPERTIES OF STARCH IN HIGH AMYLOSE POTATOES

Starch has been studied thoroughly for many decades, but there are still great difficulties to understand its properties. Even if we can define exactly which physical property we desire, we cannot predict what the structure should be to reach that goal. We also need to know more about how to modify the starch biosynthesis in order to obtain a predefined starch structure. A simple way to describe these problems would be to say that starch is build up by very complex molecules and its properties depend on a multitude of structural features. The possibility to make different changes in the branching of potato starch gave us the opportunity to broaden the knowledge on starch structure and properties. We have also found new ways to analyse the chemical structure.

NUTRITIONAL PROPERTIES OF POTATO STARCH FROM HIGH AMYLOSE POTATO

We characterized starch from three of the potato lines that had the starch branching enzyme suppressed

Using CRISPR/Cas9 we could mutate different combinations of the eight alleles coding for the two enzymes that affect the branching of the starch molecule. The high amylose traits had an expected negative effect on tuber yield, dry matter and tuber phenotype. Top: variety Desiree, without genome editing. Middle: All alleles for branching enzyme 1 knocked out plus 2-3 alleles of branching enzyme 2 mutated. Bottom: all 8 alleles coding for the branching enzymes mutated. (Photo: Ann-Sofie Fält)

through RNAi. By looking at the chain length distribution after debranching we could see that the chains in amylopectin are longer in the modified lines, a somewhat unexpected result that opened up possibilities for new starch properties and applications.

The amylopectin with extra-long chains is interesting from a nutritional point of view. When potato is cooked, the starch is gelatinised, and as it cools down to eating temperature part of the amylose forms crystals that are indigestible for enzymes in our small intestine. This is called resistant starch type III and works as a dietary fibre. Cooked potato typically contains about two per cent resistant starch type III on dry weight basis. The amylopectin also retrogrades, but this is a slow process that requires a few days in a refrigerator. Retrograded amylopectin is, however, not resistant since the chains are short and the crystals become small. In the modified potato, with the new long-chain amylopectin, the levels of crystalline amylose were similar to those of the un-modified potato, but the genetic modification resulted in a much larger formation of physiologically resistant starch. The retrograded amylopectin in the novel starch is probably partly resistant since the long chains can form crystals strong enough to resist digestion. The nutritional benefit of this is that such potato would have "slower" carbohydrates. This means that they are likely to give a lower blood glucose response after a meal.

UNIQUE STARCH STRUCTURE IN HIGH AMYLOSE POTATO

We had a closer look at the nutritional quality of the starch in the amylose potato described previously, with a focus on the resistance of the starch to enzymatic hydrolysis. A high content of amylose gives a high content of resistant starch in the cooked potato. We found that the down regulation of the enzymes had an effect on amylopectin structure. The outer chain-length of amylopectin was much longer than in the unmodified potato cultivar. This unique amylopectin has properties that are similar to amylose. After cooking, the modified amylopectin recrystallizes, and it is then not as easily split as the ordinary potato starch. It is therefore more resistant, and it takes longer time to digest. An additional analysis revealed that one extra day of cold storage further increases the resistant starch content, since amylopectin needs some time to get recrystallized. This shows that the resistant starch content was influenced both by the amylose/amylopectin ratio and by the amylopectin structure. All this is important knowledge in the design of functional starch and healthier food.

STARCH STRUCTURE IN HIGH AMYLOSE CRISPR/CAS9 POTATOES

The high amylose potato lines that we developed though CRISPR/Cas9 provided an excellent set of material for exploring the effect of each branching enzyme on starch molecular structure. As mentioned above, the edited potatoes can be divided into three groups based on how many of the alleles (gene copies in the four genomes) coding for the starch branching enzymes (Sbe) that have been mutated: Group 1 with all alleles of *Sbe1* mutated, Group 2 with all alleles of *Sbe1* as well as two to three alleles of *Sbe2* mutated, and Group 3 having all alleles of both genes mutated.

Starch in group 3, without branching enzymes mutated, showed no detectable branching and contained only amylose with long chains. It produced granules and X-ray scattering showed that the majority of the starch was amorphous, but probably with minor crystalline order. Starches in group 2 were clearly affected in their amylopectin chain length distribution, with a smaller proportion of short chains. The amylopectin chain length distribution in group 1 was surprisingly similar to the distribution in the parent. We also found some differences in the chain length distribution of amylose. This is interesting since the branching enzymes are mainly involved in the synthesis of amylopectin. It is likely that the plant compensates by producing more amylose when the amylopectin synthesis is disturbed. This apparently changed the proportions of long and short chains of amylose in ways that we not fully understand at this point. Another unexpected result was that the amylopectin structure modifications we observed in this study were significantly different from those obtained in the study where the suppression of the expression of branching enzymes was made with RNAi technology. None of the CRISPR/Cas9 produced lines contained amylopectin with extended chains.

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Menzel, C. et al. 2015. Improved material properties of solution-cast starch films: Effect of varying amylopectin structure and amylose content of starch from genetically modified potatoes. *Carbohydrate Polymers* 130: 388-397.

Zhao, X. et al. 2018. Resistant starch and other dietary fiber components in tubers from a high-amylose potato. *Food Chemistry* 251: 58-63.

ADDITIONAL RESULTS IN SHORT

NEW METHOD TO DESCRIBE AMYLOPECTIN BRANCHING STRUCTURE

Starch with well-defined structural modifications is very useful in research that aims to explain functional properties on a molecular level. Understanding of the structure-function relationships is an important step towards introducing the desired functionality in food products. Based on a relatively new concept to describe the inner branching structure of amylopectin, we have developed a simplified method to isolate and characterise amylopectin building blocks. The new method can screen a large number of starches and obtain much better statistical models to reveal more structure – property relationships. The method will be a valuable tool for our future starch research.

Zhao, X. & Andersson, R. 2020. Determination of internal structure of whole starch samples after enzyme treatments – An optimized method. *In preparation.*

GI POTATO TAKES UP MORE NITROGEN

Breeding for improved crop quality traits can affect non-target traits related to growth and resource use. We compared the growth and whole-plant nitrogen economy of two genetically modified potato lines to their non-GM parental varieties, when grown in different cultivation conditions. The genetic modification inducing high accumulation of amylose in potato tubers affected several non-target traits related to plant nitrogen economy. It increased the plant's nitrogen uptake and accumulation efficiency. Due to strongly increased plant nitrogen accumulation compared to the parental variety, the cultivation of the high-amylose GM potato will likely require higher nitrogen fertilization rates. However, starch productivity per unit land area or per soil nitrogen still will be higher in the high-amylose GM potato.

Pourazari, F. et al. 2018. Altered tuber yield in genetically modified high-amylose and oil potato lines is associated with changed whole-plant nitrogen economy. *Frontiers in Plant Science* 9: 342.

ENVIRONMENTALLY FRIENDLY PLASTICS FROM POTATOES

The modification of the potato starch increased the glucose chain length of the amylopectin molecules. Thanks to this, the starch has fibre-like properties that make it suitable as a component of a new environmentally friendly packaging material. The goal is to develop a stretchable and strong material by tailoring mixtures of plant proteins and starch, in order to produce sustainable materials for various uses, including packaging and plastic film. In preliminary studies, different variants of this new the material have been obtained: some that are relatively soft and flexible, and others that are stronger and harder. Gliadin molecules (from wheat) adopted an unusual hexagonal structure in mixtures with starch, and this structure made the material stronger.

Muneer, F. et al. 2015. Nanostructural morphology of plasticized wheat gluten and modified potato starch composites: relationship to mechanical and barrier properties. *Biomacromolecules* 16: 695-705.

Field trial showing the effect of the late blight-causing pathogen Phytophthora infestans on the variety Desiree with (right) and without (left) a resistance gene introduced from potato clone SW93-1015.

Disease resistance

Pathogens and pests can infect the entire potato plant, including stems, leaves and tubers, thus causing significant tuber yield loss. Resistance breeding is therefore key for sustaining and improving the productivity of potato. The fungal-like oomycete *Phytophthora infestans*, which causes the late blight disease, is one of the most devastating plant pathogens, worldwide as well as in Sweden. Most potato varieties need frequent fungicide spraying to control the disease. The pathogen generates yearly costs of up to one billion euro in the EU alone. We have been working with several long and short term methods to increase the resistance to late blight in potato.

RESISTANCE THROUGH GENOME EDITING

The traditional method to obtain sustainable resistance towards late blight is to introduce several classical resistance (R) genes into the plant. This is very difficult to achieve with traditional breeding. As we mentioned before, most potatoes have four genomes, something that complicates crossings. R-genes are very specific for each pathogen species, and they are often quickly overcome by evolution of the pathogen. Recently, susceptibility genes (S-gene) have been identified in for example rice, wheat, citrus, tomato, and potato. We have used the CRISRP/Cas9 system to mutate several S genes in potato, including two homologues (copies of the same gene) that have been found to reduce the levels of salicylic acid. This is known to be a main defence hormone in other plants. Using this method, we managed to mutate six different putative S-genes in all four alleles. This resulted in a potato with increased resistance. Unfortunately, some had growth defects, but two remains as agronomically interesting. The genome editing approach has the potential to generate a more general pathogen resistance than only towards late blight.

MARKER DEVELOPMENT

We have earlier found that the SW93-1015 potato breeding clone has an efficient resistance against *P. infestans* under field conditions in Sweden, in spite of a high local diversity of the pathogen. Analysis of F1 potato progenies from two individual crosses resulted in nearly 50 per cent resistant clones, from both crosses. This result suggests that the SW93-1015 clone has a gene type (allele) for this trait in one of its four sets of chromosomes. Screening of over 50 different *P. infestans* effectors (pathogen molecules that manipulate the immune system in plants) revealed a specific response to one particular gene. The expression of this gene in the potato cultivar Désirée gave rise to a race specific resistance. By using RNA sequencing analyses we designed a new DNA marker for the resistance in SW93-1015. In summary, we demonstrated the use of effector screening in practical breeding material and revealed the key resistance mechanism in SW93-1015. This shows the usefulness of integrating effector screening in a breeding program and in resistance gene cloning.

FURTHER READING

Kieu, N.P. et al. Increased late blight resistance in potato through CRISPR-cas9 mediated gene editing of susceptibility genes. *In preparation.*

Lenman M. et al. 2016. Effector-driven marker development and cloning of resistance genes against *Phytophthora infestans* in potato breeding clone SW93-1015. *Theoretical and Applied Genetics* 129: 105-115.

PROTEOMICS FOR RESISTANCE BREEDING

Proteomics means that the proteins in the cells are examined (unlike genomics, where the genetic material is examined). Just as with gene sequencing, we can use proteins as markers for selection at an early stage in the individual's development. By integrating the tissue-specific expression measurements obtained by proteomics and the genomics data for the same potato clones, it is possible to locate the chromosomal positions for the most promising markers for late blight resistance in the tubers. There are often several copies of genes present in plant genomes, and several variants of genes that are very similar to each other. This makes it difficult to predict plant characteristics from DNA, in particular in plants that are tetraploid, like the cultivated potato.

Using mass spectrometry, more specifically the technology *selected reaction monitoring*, it is possible to determine the amounts of selected peptides and proteins in a plant. First of all, it is important to find out which of these molecules are specific to the plants with good properties. Then, plant breeders can use the peptides and proteins as markers for properties such as resistance to disease, drought tolerance, and adaptation to a particular region.

The markers can thus tell the plant breeder at an early stage what properties the plants have, without having to wait for the mature plants where the properties per se can be identified. This saves energy, time and money. However, it is trickier to analyse the proteins. DNA sequences consist of four different bases, and there are easy methods for amplifying large amounts of DNA. The proteins consist of many more constituents (amino acids), and the researchers are limited by how much protein they manage to obtain from a leaf or a potato tuber. Furthermore, different proteins behave very differently. Therefore, they are more complicated to analyse than DNA.

PROTEINS CAN TELL THE STORY ON THE COURSE OF THE DISEASE

When a plant is attacked by an insect or a pathogen, an array of responses start. Some of those responses can be very specific to the attacker, and they have evolved during the evolutionary arms race between the plant species and the pest. In order to "know the enemy" we performed a large scale proteomic analysis of the pathogen causing late blight, *P. infestans*, at six life stages to identify proteins that change in abundance during development, with a focus on pre-infectious life stages. Over 10 000 peptides from over 2000 proteins were analysed. We managed to identify proteins that were up- or downregulated in different combinations of life stages, and therefore probably are of high importance for the pathogen at different life stages. We identified a group of 59 interesting proteins that were highly abundant in the pathogen at pre-infectious life stages, i. e. in its germinated cysts and the cells that penetrate into the potato plant. A large majority of these proteins had not been recognized before as involved in this infection process. Based on their similarity to other proteins, with known function, their roles in transport, amino acid metabolism, pathogenicity and cell wall structure modification could be identified.

We also analysed the expression of the genes encoding nine of these proteins. We found an increased level during disease progression, in agreement with the hypothesis that these proteins are important for the infection to occur. Some of these proteins are involved in the pathogen's struggle to modify and hold on to the cell wall structure. Silencing of these genes resulted in reduced severity of the infection, additionally indicating that these proteins are important for pathogenicity.

PROTEINS USED IN THE DEFENCE AGAINST POTATO LATE BLIGHT

We have established a system for investigation of immunity responses in potato. Using that system we have tried to find out more about which plant proteins are involved in what kinds of defence systems during the attack of *P. infestans*. Our focus was on the composition and levels of specific proteins in leaves of potato. For instance, a protein annotated as a sterol carrier protein shows high abundance in plants that are under attack. This is interesting, and not surprising, since the oomycete relies on the plant host for sterols. In addition to the sterol carrier protein, we found an increased abundance of several RNA-binding proteins. Those kind of proteins have a role in a numerous cell processes and in the post-transcriptional control of gene expression.

Plants have a wide variety of ways to defend themselves at different stages of a pathogen attack. They can recognize so-called pathogen-associated molecular patterns (PAMPs) that result in a first level of basic defence named PAMP triggered immunity (PTI), inducing the transcription of defence related genes. This PTI defence can be suppressed by crafty pathogens that secrete molecules called effectors into the plant cell. To counteract this, some plants have in their turn evolved proteins that recognize effectors and initiate a second level of defence, called effector triggered immunity (ETI). We found that some changes in protein abundance are only regulated in PTI, and not at all in ETI interactions. One such potato protein has a domain (part) that resembles a part of a protein found in barley. That barley protein is involved in regulating the plant's basic resistance. There was also an increased level of a glyoxysomal fatty acid beta-oxidation multifunctional protein in the PTI interaction. It has earlier been shown that an *Arabidopsis* mutant lacking that protein had a reduced accumulation of jasmonic acid (JA) accumulation, a hormone involved in plant defence. This is an indication that an increase in this protein might contribute to a generation of signalling molecules needed for the PTI response.

We also observed another interaction-specific change, namely that a family of catalase proteins were only upregulated in the ETI interactions. Catalase related genes have previously been found to be regulated by both biotic and abiotic stresses, for example in sugarcane during plant–pathogen interactions. A few proteins were regulated in only one of the ETI interactions, for example a number of histones, which are important in the package of DNA in cell nuclei. Our results can be hopefully be used in future potato pre-breeding to predict sustainable combinations of resistance or susceptibility genes.

FURTHER READING

Burra DD. et al. 2018. Comparative membrane-associated proteomics of three different immunity reactions in potato. *International Journal of Molecular Sciences* 19: 538.

Resjö, S. et al. 2017. Proteomic analysis of *Phytophthora infestans* reveals the importance of cell wall proteins in pathogenicity. *Molecular and Cellular Proteomics* 16: 1958-1971.

Resjö, S. et al. 2019. Proteomics of PTI and two ETI immune reactions in potato leaves. *International Journal of Molecular Sciences* 20: 4726.

Genomic prediction of breeding values

Genetic gains due to crossbreeding for productivity per se appear to be stagnant in potato, whose genetic enhancement needs to overcome inherent barriers such as tetraploidy (four sets of chromosomes), outcrossing and heterozygosity. In the potato breeding programme "Sveriges potatisförädling", about 40 crosses are made every year, aiming at 10 000 new breeding clones in the first cycle of selection. Selections in first clonal generation are based on tuber characteristics (skin finish, colour, size and shape uniformity). Tuber quality traits and host-plant resistance to *P. infestans* are more important in later cycles of selection. Tuber quality traits include cooking and frying quality, flesh colour, and density. By combining marker-based selection with estimated breeding values for both simple and complex traits, we are able to reduce the time needed in the selection stage in the breeding process. Currently, marker-based resistance breeding in potatoes is limited to only a few genes, which affect resistance to late blight, nematodes and viruses. Association genetics has provided insights onto genetic architecture and inheritance of breeding traits, as well as tagging them to DNA markers for further indirect selection. We propose an interploidy breeding approach (crossbreeding between individuals that differ in the number of sets of chromosomes) that makes use of genomic selection and gene editing for both population improvement and cultivar development. Modern potato breeding methods following a genomic-led approach provide means for shortening breeding cycles and increasing breeding efficiency across selection cycles. Acquiring genetic data for large breeding populations remains, however, expensive for small and medium size enterprises such

Genomic selection can speed up the breeding process. However, initially, large efforts are needed to collect data.

as Sveriges potatisförädling. By recording genotyping and phenotyping data in populations from their breeding programme we could investigate the usefulness of genomic estimated breeding values (GEBV) for selection. We used elite breeding material from three locations and different years to determine the GEBVs and to search for correlations between the traits and the genes (genome-wide association research, GWAS). We also reduced the number of SNPs that are needed, which lowers the cost of genotyping large populations (GWAS) or to predict GEBV for selection. This pipeline allows for creating custom SNP subsets to cover all variation found in any particular breeding population. The accuracy estimates for GEBV for selection depend on modelling methods, pedigree along with population structure, phenotyping data reliability, training (reference) population size, and target traits.

FURTHER READING
Ortiz, R. 2020. Genomic-led potato breeding for increasing genetic gains: achievements and outlook. *Crop Breeding, Genetics and Genomics* 2: e200010.

Ortiz, R. et al. 2020. Svensk potatisförädling: Breeding the new table and crisp potatoes for Sweden. *Sveriges Utsädesförenings Tidskrift* 1-2020. In press.

Increasing nitrogen up-take

Crop production efficiency relies largely on inputs of mineral nutrients, in particular nitrogen. The extensive use of such chemicals causes severe nutrient leaching into the environment, which is one of the largest environmental concerns globally. Plants' uptake of nitrogen comes both from mineral and organic sources. Considerable resources have been invested in trying to improve crop uptake of inorganic nitrogen sources, but success has been limited. The research on uptake of organic nitrogen sources, such as amino acids, has been, and is still very limited. Plants are capable of using a range of organic nitrogen sources such as amino acids and peptides which are produced via breakdown of proteins in dead organic materials and constitute the dominant forms of nitrogen in soils. Improved understanding of the mechanisms in the uptake of organic nitrogen has opened up the possibility to alter plant uptake.

The classic science on plant nutrition states that plants acquire nitrogen from the soil as nitrate or ammonium, or as nitrogen gas if the plant forms symbiosis with nitrogen-fixing bacteria. Today we know that there are plants that also can make use of organic nitrogen, but the consensus has been that it only applies to certain mycorrhiza-forming plants growing in nutrient-poor soils. Together with colleagues from Austria and Australia we found that a plant that does not form mycorrhiza acquires organic nitrogen when grown in nutritious agricultural soil.

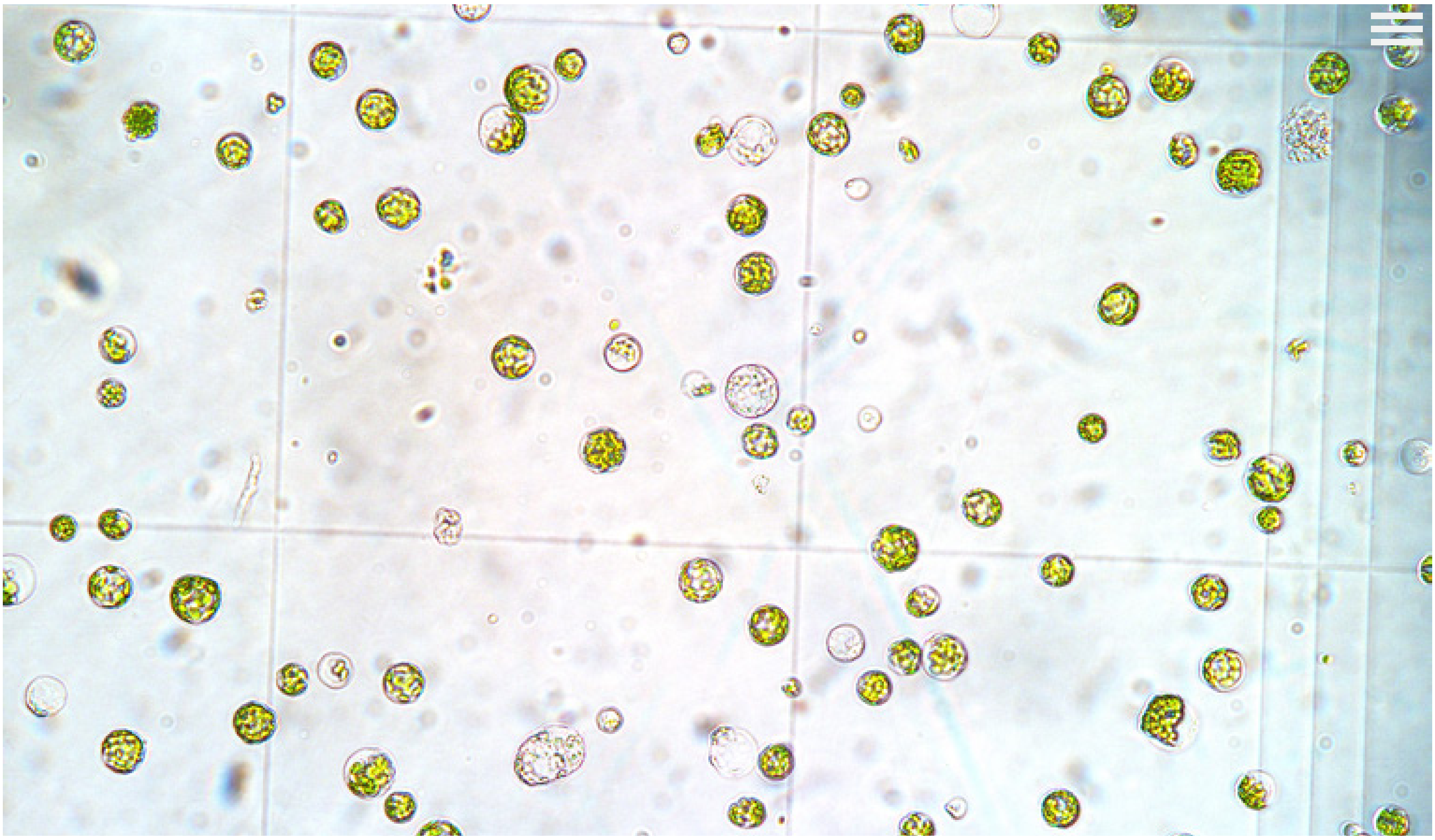
Our findings show that the plant is dependent on a specific protein for this to work. The protein is an amino acid transporter, and we performed a number of experiments on genetically modified thale cress (*Arabidopsis thaliana*) that either lacks the transporter or overproduces it. By labelling the glutamine with carbon and nitrogen isotopes we could follow the path of the amino acid from the soil into the plant. It turned out that the uptake of the amino acid is much more efficient in plants that overproduce the amino acid transporter, and very low in plants lacking the transporter. Additionally, the plants without the transporter had the lowest carbon/nitrogen ratios, and the plants overproducing the transporter had the highest ratios. Theoretically the amino acids should contribute to a higher carbon concentration, compared to an inorganic nitrogen source, which indicates that the plants have been taking up organic nitrogen from the soil continuously.

The demonstration of enhanced root uptake rates of amino acids in Arabidopsis stimulated us to study if root amino acid uptake could also be enhanced in other plants, such as potato. We have overexpressed genes encoding transporters that mediate root uptake of various amino acids in potato, and shown that lines overexpressing such transporters in general have higher uptake rates than the wild type line. However, the results have not been consistent. One reason may be that potato roots grown *in vitro,* independently of genetic background, display large morphological differences, factors that influence the uptake.

FURTHER READING
Ganeteg, U. et al. 2017. Amino acid transporter mutants of Arabidopsis provides evidence that a non-mycorrhizal plant acquires organic nitrogen from agricultural soil. *Plant, Cell & Environment* 40: 413–423.

ADDITIONAL RESULTS IN SHORT

SITE-DIRECTED MUTAGENESIS
TECHNIQUES FOR TALEN AND CRISPR
When the Mistra Biotech program started, the hot new site-directed mutagenesis technique was TALEN. We managed to establish this genome editing technique for tetraploid potato through transient TALEN expression



Editing the genome of a plant requires that you manage to get the "editing tools" inside the plant cell. One way is to remove the cell wall while keeping the cell alive, add the "editing tool" to the remaining protoplast, regrow the cell wall, and then make the cell duplicate and grow into plant tissue. (Here field cress protoplasts.) (Photo: Sjur Sandgrind)

in protoplasts (cells with the cell wall removed). The frequency of successful site-directed mutation was 7–8 per cent, with a few base deletions as the predominant type of mutation. Among the calli (groups of unorganized cells) grown from the protoplasts, 11–13 per cent showed mutations. A similar frequency (10 per cent) was observed in the regenerated shoots. This protoplast protocol was later on successfully used in potato in modifying other traits, like starch quality, both in our research environment and other research centres in the world.

Nicolia, A. et al. 2015.Targeted gene mutation in tetraploid potato through transient TALEN expression in protoplasts. *Journal of Biotechnology* 204: 17-24.

PROTEIN NAVIGATION IN PLANT BREEDING

We developed methods for analysing plant proteins that can be used in potato breeding. We investigated 104 protein markers. Some of these could be used to predict traits such as high yield, and resistance against late blight. These are traits for which there are currently no commercial DNA markers available. We also suggested a new workflow, including both DNA and protein analyses, to accelerate the breeding for important crop traits.

Chawade, A. et al. 2016. Targeted proteomics approach for precision plant breeding. *Journal of Proteome Research* 15: 638-646.

FIELD-OMICS – UNDERSTANDING LARGE-SCALE MOLECULAR DATA FROM FIELD CROPS

The recent advances in gene expression analysis as well as protein and metabolite quantification make it possible to capture complex biological processes at the molecular level in field trials. In this way, we can study the complexity of field systems and shed light on the "black box" between genotype and environment. With solanaceous species as an example we put forward the term field-omics. Field-omics strives to couple information from genomes, transcriptomes, proteomes, metabolomes and metagenomes to the long-established practice in crop science of conducting field trials. With field-omics we can adapt current strategies for recording and analysing field data to facilitate integration with '-omics' data.

Alexandersson, E. et al. 2014. Field-omics -understanding large-scale molecular data from field crops. *Frontiers in Plant Science* 5: 286.

POTATO AS A MODEL FOR FIELD TRIALS TO STUDY MODIFIED GENE FUNCTIONS

Gene technology and genome editing are not only biotechnological techniques for creating new crop varieties but also tools for researchers to discover gene functions. Field trials following laboratory experiments are an important step in order to evaluate new functions since many phenotypes, and combinations thereof, are difficult to detect in controlled environments, and molecular analysis is nowadays possible to do in the field. We described a standard protocol for creating new potato lines and producing seed tubers for field trials within one year.

Kieu, N.P. et al. Potato as a model for field trials with modified gene functions in research and translational experiments. In *Methods in Molecular Biology*. Springer, book series. *Accepted.*

Potato defences against early blight *Alternaria solani*
Salicylic acid (SA) and jasmonic acid (JA) are the two most common signalling substances in plants, but their pathways and functions in different plant responses are complex. We found that SA is necessary to restrict *Alternaria* growth and early blight symptom development in both tubers and foliage of potato. This result is in contrast to the documented minimal role of SA in resistance to necrotrophs (organisms feeding on dead plant tissue) in the model plant *Arabidopsis thaliana*. We also investigated the gene expression during infection. Taken together, these results indicate that unimpaired SA signalling, but not JA signalling, is required for potato defences against the necrotrophic pathogen *A. solani*.

Brouwer, S.M. et al. Intact salicylic acid signalling is required for potato defences against the necrotrophic causal agent of early blight *Alternaria solani*. *Plant Molecular Biology*. *Accepted.*

HOST PLANT PREFERENCE AFFECTED BY BLIGHT RESISTANT TRANSGENIC POTATO

Introduction of major resistance genes against *P. infestans*, from wild *Solanum* species into potato can change the plant-pathogen interaction dynamics, but little is known about the effects on non-target organisms. Thus, we examined the effect of *P. infestans* itself and introduction of a resistance gene into the crop on host plant preference of the generalist insect herbivore, *Spodoptera littoralis*. Only when plants were inoculated a difference was seen and the insects laid fewer eggs on the resistant than on the unmodified susceptible plants. Other life stages of the insect were not affected.

Abreha, K.B. et al. 2015. Inoculation of transgenic resistant potato by *Phytophthora infestans* affects host plant choice of a generalist moth. *PLOS One* 10: e0129815.

THE MOLECULAR MECHANISMS BEHIND THE PLANTS´ DEFENCE

Plant resistance inducers (PRIs) are agents that give rise to improved protection to pathogen attacks by inducing the plant's own defence mechanisms, so called induced resistance (IR). PRIs are known to be effective against various pathogens (viruses, bacteria, oomycetes and fungi) attacking solanaceous plants. We have summarized the PRIs that have been successfully used in the *Solanaceae* plant family and present findings on the molecular processes that take place after application of PRIs. In general, there is a lack of consistency in the efficiency of IR both between and within solanaceous species. In many cases, a hypersensitivity-like reaction is needed for the PRI to be efficient. We predict that PRIs can play a role in future plant protection strategies in *Solanaceae* crops if they are combined with other means of disease control.

Alexandersson, E. et al. 2016. Plant resistance inducers against pathogens in Solanaceae species – from molecular mechanisms to field application. *International Journal of Molecular Sciences* 17: 1673.

NOVEL METHOD FOR BLACKLEG DISEASE SCREENING OF *IN VITRO* GROWN POTATO

Blackleg disease is a long standing and intractable problem that gives rise to increasing yield losses in Europe. It is caused by several different soft rot bacteria, one of which is *Dickeya solani*. Recently, this pathogen has been spreading in Northern Europe, and there seem to be no resistant commercial potato varieties. It is complicated to test for due to strong environmental influence on disease development, and there is currently a lack of efficient testing systems. We describe a system for quantification of blackleg symptoms on shoots of sterile *in vitro* potato plants, which saves time and space compared to greenhouse and existing field assays. This system facilitates efficient screening of blackleg disease response of potato plants independently of other microorganisms and of variable environmental conditions. We also used the screening method to study the plant mechanisms involved in blackleg disease, and show that both JA and SA pathways regulate tolerance to blackleg disease in potato, a result unlike previous findings in *Arabidopsis* defence response to necrotrophic bacteria.

Burra, D.D. et al. 2015. Salicylic and jasmonic acid pathways are necessary for defense against *Dickeya solani* as revealed by a novel method for Blackleg disease screening of *in vitro* grown potato. *Plant Biology* 17: 1030-1038.

Cereal crops for the Nordic climate

Barley has a long history in the Nordic countries and was the main cereal until other cereals gained in popularity. Its wide adaptability to different conditions has made it possible to develop varieties adapted to different vegetation periods and soil types. But there are several pathogens that can cause problems. Early infestations of leaf blotch (*Pyrenphora teres*, also called *Drechslera teres*) can reduce the harvest by up to 20 per cent, which is why we took an extra interest in this disease and how to introduce resistance towards it. We have also investigated the potential to increase the nitrogen up-take of barley, which could be environmentally advantageous.

Next to winter wheat and spring barley, oats is the main cereal crop in Sweden. Oats contain components such as dietary fibre, lipids, b-glucan and avenanthramides, which are nutritionally advantageous. The breeding goals include, as for all crops, good quality products and adaptation to environmental challenges and stresses such as drought and diseases. Key to successful cultivation of oats in Scandinavia is management of Fusarium head blight (FHB), a disease caused by *Fusarium graminearum*. In addition to causing yield losses and reduced seed germination, several *Fusarium* species produce mycotoxins, which can have detrimental effects on human and animal health. We have investigated whether genomic selection can be used to support resistance breeding in oats.

An environmentally friendly barley

Growing crops more effectively and get higher yields with lower or sustained inputs goes hand in hand with lower emissions from agriculture. How can crops be tailored to take up nitrogen more efficiently? One strategy is to develop varieties with a denser and deeper root system. Another strategy is to use biotechnology to alter the activity of the enzymes that affect the uptake of nitrogen in plants. In the absence of commercial nitrogen efficient crops and long-term field trials, we have used three simulation models to estimate the effects of an introducing barley varieties with improved nitrogen uptake. The simulations were based on conditions typical for barley cultivation in southern Sweden, as well as in areas close to the lakes Hjälmaren and Mälaren.

A barley variety that takes up more nitrogen compared to the varieties currently used would be more climate friendly thanks to 1. a higher yield, 2. an increased storage of carbon in the plants due to more biomass, and 3. a reduced emission of the greenhouse gas nitrous oxide. Our study indicates that, these effects would indeed take place, and that they would be largest in the southern parts of Sweden.

There may be a risk that a crop with a higher nitrogen uptake could result in farmers using more nitrogen fertilizer, however, that does not mean that it would result in increased nitrogen leaching. But if a crop is very efficient in taking up nitrogen initially, it might suffer from nitrogen deficiency later on if more nitrogen is not added.

We tried to put this into application by introducing two amino acid transporter genes into barley to improve the nitrogen use efficiency. The lines were tested in greenhouse and field, however the amino acid uptake was not clearly affected.

When evaluating the environmental impact of a crop, we should focus more on what is returned to the soil after we have taken out what we wanted, in this case the grain, and emphasize the total biomass, not only the grain yield.

FURTHER READING

Tidåker, P. et al. 2016. Estimating the environmental footprint of barley with improved nitrogen uptake efficiency - a Swedish scenario study. *European Journal of Agronomy* 80: 45-54.

Bombardment technique for CRISPR breeding in barley

Many diseases in barley have been partly controlled by resistant varieties from conventional breeding programmes, but the resistance is often overcome by pathogen evolution in less than five years. One alternative approach would be to block so-called susceptibility (*S*) genes. The leaf blotch pathogen is necrotrophic, which means that it actively kills host tissue to thrive on the dead or dying cells. This becomes problematic in plants that defend themselves by killing off their own cells to stop the infection (Scorched earth). However, the plant needs specific proteins to be able to recognize that it is being attacked. The genes coding for such proteins are called susceptibility genes (S-genes). If an S-gene is knocked out, the plant will become resistant against pathogens recognised by that particular protein. We have focused on one candidate *S* gene, which is upregulated in susceptible barley cultivars when infected by leaf blotch. To knock out this gene, we first cloned the gene from the barley cultivar Rika and constructed CRISPR vectors, followed by particle bombardment to deliver the CRISPR vectors into the plant cells. (Barley is very difficult to infect with *Agrobacterium tumefaciens*, which is usually used to deliver genetic material.). We first regenerated shoots from bombarded embryonic calli without antibiotic selection in order to get mutation lines without external DNA integration, but no mutation lines were confirmed out of 200 regenerates. We then shifted to cultivate the bombarded embryonic calli with antibiotic selection. This work is still ongoing. It has been technically difficult to obtain mutation lines in barley.

Goals and hurdles in genomic selection in crops

Genomic selection aims to use genetic markers across the whole genome as a selection tool in breeding programmes. It is a so-called "black box" approach since statistical associations rather than knowledge of the biological functions of genes are used. The method has been developed from previous marker-based techniques and has become more useful in recent years as genotyping has become more affordable.

Today this method has been integrated into dairy cattle breeding programmes and is being tested in other livestock and in plant species. However, each crop species has its own specific setting, and the application of strategies based on genomic selection is often far from easy. We have summarized the goals and hurdles for a successful implementation of genomic selection in breeding programmes for rice, maize, wheat, barley, and forage grass, i. e. both annual and perennial crops.

Future studies should be more targeted towards breeding programmes and should be specific for different populations (e.g. self- or cross-pollination). Crop breeding can also be improved by further advancement of methods that include environmental factors, especially genotype-by-environmental interactions. We conclude that an open exchange on the status of research results and achievements in breeding programmes is required to achieve significant success, and that collaborations with the industry is needed, since relevant findings can only come to use via the work of industry partners.

FURTHER READING

Jonas, E., & de Koning, D.J. 2013. Does genomic selection have a future in plant breeding? *Trends in Biotechnology* 31: 497–504.

Genomic selection in oats with a special focus on *Fusarium*

An increase in the incidence of Fusarium head blight and in the occurrence of mycotoxins has been observed in oats in Sweden and Norway, and the potent toxin deoxynivalenol (DON) producing species *Fusarium graminearum* has become more common. In oat breeding programmes some lines have been identified being as susceptible or resistant against this fungal pathogen. While many studies have been conducted in wheat or barley, very little is known about which parts in the oat genome linked to *Fusarium*. This is difficult to study because of large environmental effects that lead to large differences in the level of infection between different years. We have performed an association analysis using genome-wide SNP markers, which made it possible to identify a number of QTLs on different chromosomes connected to the level of DON mycotoxin. We verified three previously identified chromosomes containing QTLs and identified three novel loci on chromosomes associated with mycotoxin contents in oats. These results make it possible to identify regions for the selection of resistant lines. However, we could not identify identical regions across multiple years and for both countries. The QTL regions that we identified can be compared to those previously found in wheat. Two strong candidate genes in wheat have also been identified in oats. But the insufficient information on the whole genome sequence from oats and lack of comparative maps between wheat and oats make it difficult to clearly align the regions from wheat and oat. However, more information on resistance in the Nordic population will be needed, for instance in order to determine if the ranking of lines according to DON levels) differs between Norway and Sweden. Further analysis of the combined effects of genotype and environment are also required. Strong QTLs will then have the potential to be integrated into selection strategies to identify the most valuable lines for future breeding. Further discussions with geneticists at Lantmännen and Graminor suggested the readjustments of models. We have now corrected repeated data using spatial analysis to take the replications within each year and environment as well as the geographical location into account. We are currently testing the predictive ability of the data from one dataset for the other datasets, using different sets for reference populations. We will also test if the newly adjusted data will improve the identification of QTL regions for DON contents. If additional genotypic and phenotypic data becomes available from the companies in the future, this will also improve the analysis of the data.

In addition to causing yield losses and reduced seed germination, several Fusarium species also produce mycotoxins, which may make the oat unsuitable as food or feed.

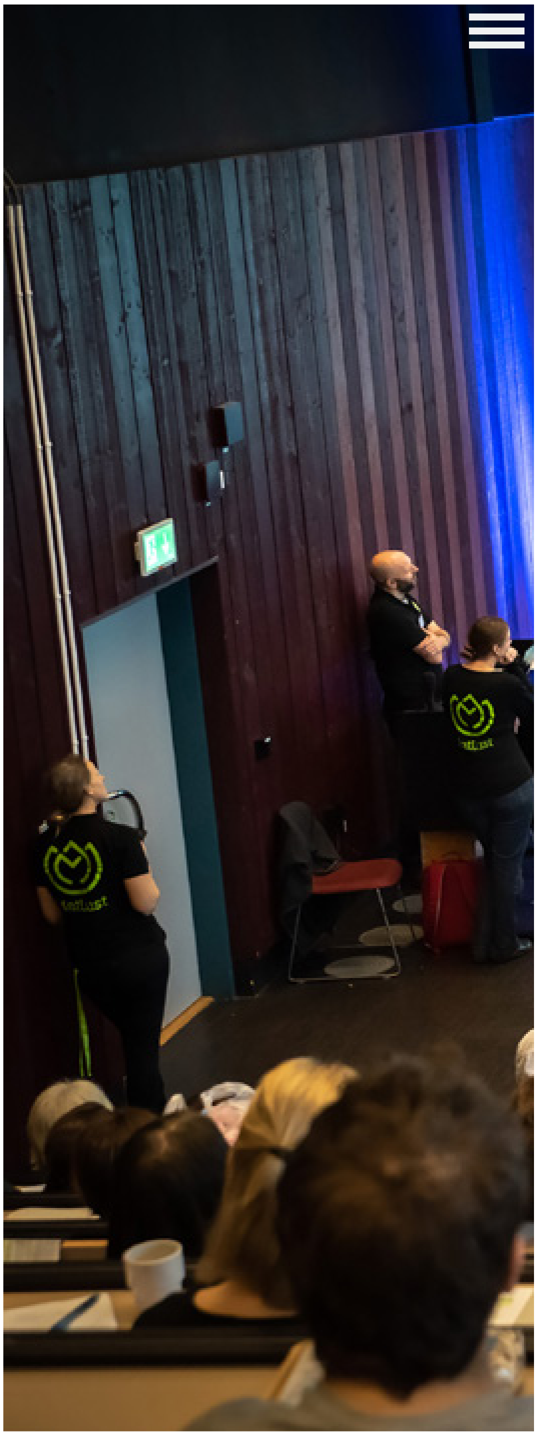
Simulated breeding programme for oats

We have developed a full simulation of a breeding program to mimic the breeding program of oats, an inbred crop. The simulation allows us to gain information on genotypes (or genetic marker alleles) and the derived phenotypes, based on defined QTLs in the genome. In applied breeding schemes, information on actual phenotypes is not available during the first cycles of breeding and selfing, but when it becomes available, such information will be useful for deciding the best strategies for genomic selection. When the most diverse, highly resistant, and very susceptible lines for DON are identified for the first crossings within the simulated breeding program, then that the information can be used to re-run the simulation. The QTL information identified in the real data can be integrated into the simulation, which will give more realistic phenotypes (closest to real DON values). Thereafter the genetic merit of specific lines can be estimated to test different cross validation schemes based on the data achieved. While our current simulation based on random QTLs suggested that predictions work very well, validation based on more reliable phenotypic data is still required.

FURTHER READING

Jonas, E. et al. Genome-wide association analysis for DON content in oats tested in two environments during multiple years. *In preparation.*

Jonas, E. *et* al, A. Simulation of breeding program of an inbred crop for the cross validation of genomic breeding values across generations. *In preparation*

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Communication

Highlights from our internal and external communication efforts from 2012 to March 2020.

Eight years of science communication

"– Why are you doing this?! Nobody wants it!", was the message I received in a telephone call before Mistra Biotech had even officially started. It was clear that the words 'biotechnology' and 'sustainable' in the programme description (Biotechnology for sustainable and competitive agriculture and food systems) was not something the caller saw as compatible or worthy of investigating, a view that was shared by many.

At the same there was a sense of expectation from those in the plant breeding and research community who wanted us to try to succeed where others up to that point had "failed". They hoped Mistra Biotech communications would change the minds of people who did not want GMOs, but changing public perception was never the programme's goal.

Our aim was simply to provide society with the solid scientific facts that people need to form an informed opinion, whatever that opinion might be.

The areas of biotechnology, plant and livestock breeding involve so many aspects, from technical, biological and agronomical questions, to social, ethical and economic issues, that the debates often get lost in a jumble of arguments. There is also a fair share of myths, misunderstandings, and distrust in this area, which further complicate the issue. Building trust has been a key component in our communication efforts, since developing trust is crucial if you wish to create a meaningful dialogue with a broad audience. Thus, foremost on our agenda was to be perceived as a trustworthy knowledge hub. To achieve this we needed to let our research results speak for themselves and avoid drawing, or presenting, any opinion-based conclusions, or reflection, on the research itself.

Early on in Mistra Biotech, it was also evident that the benefits from breeding crops and livestock – with or without biotechnology – are very unclear to most people. In fact, not many people are aware of the gigantic changes our crops and livestock have gone through during domestication, and how those changes have improved human life. Before talking about the pros and cons of using a certain breeding technology in our food production, it is important to clarify the purpose of breeding in the first place. Otherwise, the communication will not be meaningful.

Over the eight years that have passed since the start of the programme, we believe that there has been a change in how gene technology is discussed both in Sweden and globally. In the comments sections of blog posts and news articles on the web, there seems to be a shift to a more nuanced approach where people distinguish between different types of genetic modification. The development and application of genome editing, such as CRISPR/Cas9, has also blurred the boundary between GMO and non-GMO, making some of the arguments against biotechnology outdated. We also believe that the way many researchers communicate their science has changed over the past years, with more emphasis on two-way communication.

Our hope is that the future debate on the usage of biotechnology in agriculture and food production will remain based on science, but communicated a way that makes the debate accessible to all and addresses the concerns of different segments of our society. This means taking into consideration the different levels of scientific knowledge and understanding, as well as the range of different perspectives and concerns the wider society has.

I will leave you with the words of Professor Dame Anne Glover, Chief Scientific Adviser to the President of the European Commission from 2012 to 2014, who unintentionally summarized everything we have tried to achieve through Mistra Biotech communication when she said: "We need to be honest about the facts we have and don't have, and we need to show empathy for public concerns….We need to offer options for action, rather than claiming to have the ultimate solution.".

Anna Lehrman
Deputy programme director and Communications officer

Lisa Beste and Anna Lehrman have been the hosts of the podcast Shaping our food that Mistra Biotech launched in August 2018.

SHAPING OUR FOOD

The podcast

Shaping our food, a podcast produced by Lisa Beste and Anna Lehrman has been one of our main channels for presenting the research in Mistra Biotech to the public, along with input from external researchers and experts. Up until June 2020, it has had over 4500 downloads. The following episodes have been published. (The episodes are presented in English here although the podcast is in Swedish. Affiliations and titles refer to the status at the time the episode was recorded).

1. **Why do we need plant breeding?** Guest: Inger Åhman, professor in plant breeding at SLU.
2. **Why do we need animal breeding?** Guest: Lotta Rydhmer, professor in animal breeding at SLU.
3. **Taming a wild plant.** Guests: Emelie Ivarson, PhD student, Sten Stymne and Li-Hua Zhu, both professors in plant breeding at SLU.
4. **Fighting the blight!** Guest: Erik Andreasson, professor in plant protection at SLU.
5. **Now it is time for oats.** Guests: Alf Ceplitis, senior breeder and technology manager at Lantmännen, Elisabeth Jonas, associate professor in quantitative genetics at SLU, and Olof Olsson professor in applied biochemistry and project leader at ScanOats, Lund University.
6. **One hundred per cent unnatural.** Guest: Per Sandin, associate professor in philosophy at SLU.
7. **Green fields, not green oceans.** Guests: Henrik Svennerstam, researcher at Umeå Plant Science Centre and Martin Weih, professor in plant ecology and eco-physiology of agricultural crops at SLU.
8. **Proteins take us closer to truth.** Guest: Fredrik Levander, associate professor in immuno-technology at Lund University.
9. **Noah's ark for real – gene banks save traits for the future.** Guests: Ulrika Carlson-Nilsson, senior scientist and Pawel Chrominski, *in vitro* lab responsible at NordGen, and Denise Costich, senior scientist and head of the germplasm bank, and Bibiana Espinosa research associate at CIMMYT, the International Maize and Wheat Improvement Center in Mexico (interviews in English).
10. **Creamy, greasy, but still stable – this is starch.** Guests: Mathias Samuelsson, sales and development director at Sveriges stärkelseproducenter (the Swedish potato starch cooperative), and Mariette Andersson, researcher in plant breeding and Roger Andersson, professor in food science, at SLU.
11. **Genes by all means – from genomics to traits.** Guests: Cecilia Gustafsson, geneticist in genomics-assisted plant breeding, and Dirk-Jan de Koning, professor in animal breeding, at SLU.
12. **Thousands and thousands of varieties – but only one GMO.** Guests: Jens Weibull, senior officer at the Swedish Board of Agriculture, Marie Nyman, chief secretary at the Swedish Gene Technology Advisory Board, and Dennis Eriksson, researcher in plant breeding at SLU.
13. **Fifty per cent like mum, sixty per cent like a banana?** Guests: Fredrik Sundström, director of studies at the Biology Education Centre at Uppsala University, and Niclas Gyllenstrand, curator at the Swedish Natural History Museum.
14. **Consumers, food, and gene technology.** Guests: Minna Hellman, manager, consumer health and well-being, Stockholm Consumer Cooperative Society, and Carl Johan Lagerkvist, professor in business economics at SLU.
15. **GMO + organic = true?** Guests: Lars Hällbom previously standards director at KRAV, Minna Hellman, manager, consumer health and well-being, Stockholm Consumer Cooperative Society, Sara Sundquist, sustainability manager and industrial policy expert at the Swedish Food Federation, and Carl Johan Lagerkvist, professor in business economics, Klara Fischer, associate professor in rural development, and Per Sandin, associate professor in philosophy, at SLU.

16. **Artificial intelligence at the breeder's service.** Guests: Aakash Chawade, associate professor in plant breeding at SLU, and Tina Henriksson, senior breeder at Lantmännen.
17. **"Yes, and by the way no" How a whole industry changed their minds on GMO.** Guest: Bo Ekstrand, CEO at Bioconsult AB.
18. **Ethics, animal breeding and technology.** Guest: Helena Röcklinsberg, university lecturer in animal ethics at SLU.
19. **Methods and techniques in plant breeding.** Guests: Cecilia Gustafsson, geneticist in genomics-assisted plant breeding, Jonas Skytte af Sätra, PhD student, Emelie Ivarson, research engineer, and Mariette Andersson, associate professor in plant breeding, all at SLU.
20. **How cautious should we be?** Guest: Sven Ove Hansson, professor emeritus in philosophy at KTH Royal Institute of Technology, and programme director of Mistra Biotech.

Fredrik Sundstrom, Uppsala University, and Niclas Gyllenstrand, the Swedish Natural History Museum are interviewed by Lisa Beste about how DNA is compared within and between species during a live-pod at the science fair SciFest in Uppsala 2019. (Photo: Natalie von der Lehr)

HIGHLIGHTS

Seminars and events

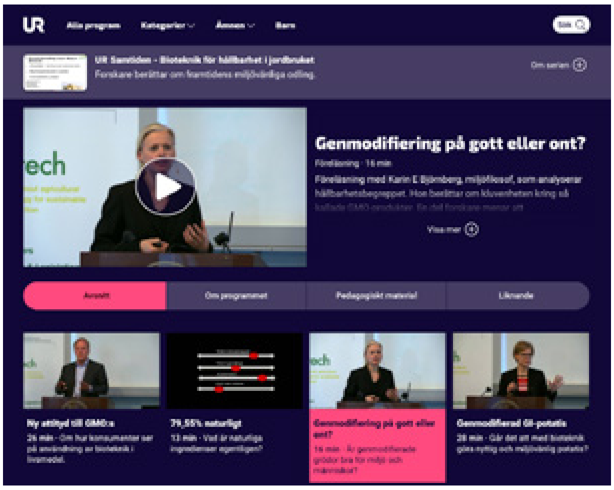
Over the years we have arranged and been part of numerous seminars, workshops, meetings and public events, apart from our many contributions at scientific conferences around the world.

Annual meetings for all associated researchers in Mistra Biotech have been important for the interdisciplinary collaborations, but also a chance for researchers who are usually geographically separated to meet in person. A "speed dating" kick-off at each meeting proved to be a successful way to remind the researchers that they have to present their work in a more popularised way when talking to someone from a different discipline.

The guided field tours started in 2012 with an event for the press in collaboration with PlantLink, TC4F and ICON. Since then we have invited the public to come and see and learn about the field trials with potato lines bred for leaf blight resistance and altered starch quality, as well as the domestication of field cress. In 2019 the field tour was combined with a survey to study if such events affect the participants' views on plant breeding and gene technology.

We have invited high school students to lectures and campus tours, and also been invited to present our research at schools and at the Senior University. Here you can see Lotta Rydhmer, Deputy programme director during phase I, explaining animal genetics and breeding to high school students in 2014.

The Mistra Biotech symposium "Biotechnology for agricultural sustainability" at the Royal Swedish Academy of Agriculture and Forestry (KSLA) in 2016 was filmed by the Swedish Educational Broadcasting Company (Utbildningsradion, UR), a public-service corporation dedicated to serving the needs of the Swedish general public by providing educational programming on radio and television. The presentations by Dirk Jan de Koning, Karin Edvardsson Björnberg, Li-Hua Zhu, Mariette Andersson and Pernilla Tidåker are available on the UR homepage. Deputy programme director Anna Lehrman participates in an episode about Golden Rice in another series from 2019, Briljanta forskare (Brilliant scientists) that brings up current research highlighting societal challenges globally, and how we seek solutions to the problems.



UR Samtiden - Bioteknik för hållbarhet i jordbruket
Forskare berättar om framtidens miljövänliga odling.

Genmodifiering på gott eller ont?
Föreläsning · 16 min
Föreläsning med Karin E Björnberg, miljöfilosof, som analyserar hållbarhetsbegreppet. Hon berättar om kluvenheten kring så kallade GMO-produkter. En del forskare menar att

Ny attityd till GMO:s
26 min · Om hur konsumenter ser på användning av bioteknik i livsmedel.

79,55% naturligt
13 min · Vad är naturliga ingredienser egentligen?

Genmodifiering på gott eller ont?
16 min · Är genmodifierade grödor bra för miljö och människor?

Genmodifierad GI-potatis
28 min · Går det att med bioteknik göra nyttig och miljövänlig potatis?

Panel discussion at the seminar day "The Future of Plant Biotechnology in Europe" organised in collaboration with Plant Link and Partnerskap Alnarp in 2012. Tina d'Hertefeldt (Lund University), Sven Ove Hansson (Mistra Biotech), Huw Jones Rothamstead (Res./EFSA), Jan Eksvärd (LRF), and Frank Hartung (JKI/EPSO).

Lunch seminar arranged in in collaboration with Future Agriculture in 2013. Nicholas Kalaitzandonakes (University of Missouri) talked about "The evolving structure of the global agrifood biotech industry and implications for future innovation".

Paula Persson, Pernilla Tidåker, Karin Edvardsson Björnberg, and Nils-Ove Bertholdsson at the workshop "Sustainability in future food production systems: Can biotechnology make a difference?" organised by Mistra Biotech in 2013.

Panel discussion during the workshop "Regulatory challenges for agricultural biotechnology in the EU" in 2014. Christopher Ansell (UC, Berkeley), Programme Director Sven Ove Hansson, Carl Johan Lagerkvist (SLU), Marie Nyman (The Swedish Gene Technology Advisory Board), and Carina Knorpp (Swedish Ministry of Rural Affairs).

In the foreground Peter Sylwan, moderator during a seminar about field cress at KSLA in 2019. Li-Hua Zhu (not in the picture) and Sten Stymne (both SLU), Magnus Edin (Sunpine), Hulda Wirsén (The Rural Economy and Agricultural Societies Norrbotten-Västerbotten), and Urban Emanuelsson (SLU) all gave presentations.

In 2013 we arranged a workshop "Ethical issues in new biotechnology in agriculture" as a satellite event to the 11th Congress of the European Society for Agricultural and Food Ethics. Here PhD student Payam Moula discusses with invited speaker Helena Siipi (Univ. of Turku).

Helen Sang (The Roslin Institute, University of Edinburgh), one of the keynote speakers, Jenny Carlsson and Marie Nyman (The Swedish Gene Technology Advisory Board) at the evening mingle during the two-day Mistra Biotech symposium and workshop "Breeding genetically modified animals for food production" in 2014.

We have organized a number of events at the Almedalen week in Visby. In 2016 a seminar "Future food in a Nordic climate – can we do it without domestic plant breeding?" with researchers Mariette Andersson, Carl Johan Lagerkvist, and Inger Åhman, politicians and stakeholders (bottom left). In 2017 a discussion seminar "Technology neutrality in practice – giving chance to modern biotechnology in Swedish and European plant breeding" with Kristofer Vamling (The Swedish Board of Agriculture) and Zofia Kurowska (previously Swedish Food Agency) among others (bottom right). (This seminar can be viewed on YouTube.) In 2019 we organized two events, a live podcast about consumers, food and gene technology, and a discussion seminar about GMO and organic food with Lars Hällbom (previously at KRAV), Klara Fischer (SLU), Sara Sundquist (The Swedish Food Federation), Per Sandin and Carl Johan Lagerkvist (both SLU), and Minna Hellman (Stockholm Consumer Cooperative Society) (top). Both events are available as episodes in the podcast Shaping our food.

The "GMO-buffet" has been presented at several events to spark discussion and give food for thought. Which products contain GMOs, and which are produced with the help of GMOs? Note that the "CRISPR-kale" in the picture was served before the EU Court decision that also genome edited crops should be considered to be GMO.

In 2017 the documentary "Food Evolution" was released and we organized a screening at SLU Ultuna campus. Before and after the screening the audience could participate in a digital survey with questions about topics in the film, followed by a discussion on both the survey results and the film itself.

Deputy programme director (phase II) Anna Lehrman was keynote speaker together with Odd-Gunnar Wikmark (GenØk – Senter for biosikkerhetpresenting), Ulf Sonesson (RISE), and Line Gordon (Stockholm Resilience Centre) (in the middle of the photo Helena Nordlund, project leader for MatLust), at the conference "Food systems in change: new technology or behavioural changes?" arranged by Matlust at KTH Södertälje Science Park during their science week in 2020. (Photo: Fredrik Sederholm)

We have participated at several of SLU's events Matologi and in the SLU stand at Borgeby agricultural fair. Researche Cecilia Gustafsson was one of several Mistra Biotech researchers who made short presentations in the SLU tent at Borgeby in 2019 (top). At Matologi in 2018 Per Sandin was one of the panellists in the discussion on the "political food choice" in the main tent, and PhD student Catja Selga and Cecilia Gustafsson answered questions from visitors in the Mistra Biotech stand (bottom).

Jean-Eric Paquet (The Commission's Director-General for Research and Innovation) – with entourage discussed CRISPR with researcher Mariette Andersson and Mistra Biotech Board member Stefan Jansson at the "FOOD 2030 High level event: Research and Innovation for Food and Nutrition Security – Transforming our food systems" in Plovdiv, Bulgaria in 2018. (Photo: Kate Wilson)

Researcher Klara Fisher was invited as a speaker at the open meeting on "GMO in Africa" arranged by the Norwegian Biotechnology Advisory Board in Oslo in 2018.

Dennis Eriksson, Mistra Fellow at EPSO, organized a workshop on plant breeding in the European Parliament in 2015.

Mariette Andersson was invited to "Innovation in agriculture: Women pioneers at the frontiers of science" held at the European Parliament, organized by the United States Mission to the European Union together with the European Parliament, S&D and EPP in 2018. (Photo: Raquel Izquierdo de Santiago)

Sven Ove Hansson was invited as a speaker at the seminar "Sustainable agriculture – does it need modern biotech?" in 2012 at the Royal Swedish Academy of Agriculture and Forestry. An example of the contributions our researchers have made at science academies over the years.

SHAPING OUR FOOD

The book

A book "Shaping our food – an overview of crop and livestock breeding", written by researchers from different research areas of Mistra Biotech, was published online in 2014 both in English and Swedish (also printed in Swedish with the title "Framtidens mat – om husdjursavel och växtförädling"). The book homepage www.slu.se/shapingourfood, where the e-version is free to read online or download as a pdf, has had up to 1600 page views per year. We have also received several requests from researchers, students and others for permission to use illustrations from the book in reports, presentations and educational material (the illustrations are made by Fredrik Saarkoppel). A new shortened edition is currently being published, and the printed version (in Swedish) will be distributed to schools through utbudet.se.

Homepage, newsletter & Twitter

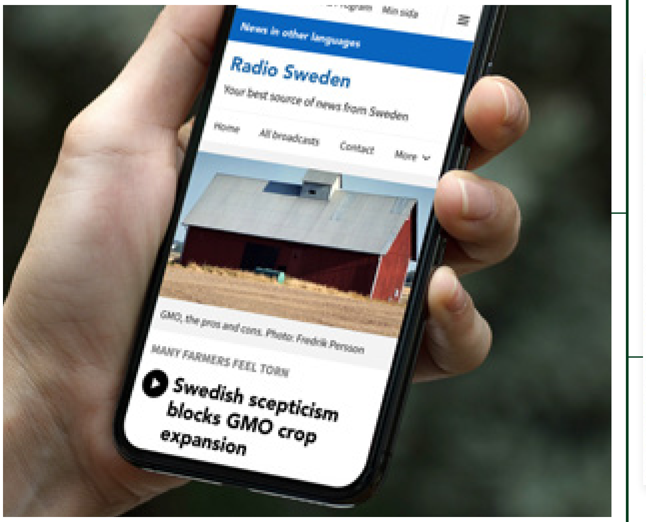
Our web had over 20 000 page viewings in 2019, with one page having over ten per cent of those: The page describing how plant breeding works. The webpage presents texts and illustrations about breeding methods from the book Shaping our food – an overview of crop and livestock breeding (Framtidens mat – om husdjursavel och växtförädling). Not far behind is the "sister page" that describes livestock breeding in the same way (those pages are only available in Swedish). By subscribing to our news feeds on the webpage it has been possible to get continuous updates on both the research in Mistra Biotech and national and global news and debates on topics related to biotechnology and crop and livestock breeding.

One channel for both external and internal communication has been the digital newsletter. The external newsletter, available both in Swedish and English, has had over 1100 subscribers from universities, industries, authorities, NGOs, politicians and students, both in Sweden and abroad. We have also communicated our research via Twitter and the Facebook podcast page. Relevant information from the Mistra Biotech web will be available through the SLU Future Food webpage.

The annual reports summarize the progress of the research and present researchers in the programme, along with invited guest writers.

HIGHLIGHTS

Media coverage



GMO, the pros and cons. Photo: Fredrik Persson

MANY FARMERS FEEL TORN

Swedish scepticism blocks GMO crop expansion

March 2012

Fem GMO-projekt för att skapa förnybara råvaror och hållbarare jordbruk

Hur möter vi framtidens hot om för lite mat på jorden och vad ska ersätta fossil olja och kol? Forskare från SLU genomför fem olika fältförsök i Skåne med korn, potatis och oljekål. Försöken görs inom området växtbioteknik.

Jordbruket står inför en dubbel utmaning: att både fördubbla livsmedels tillgången och ersätta fossil olja och kol. Modern växt-

September 2012
[Five GMO projects to create renewable raw materials and a more sustainable agriculture]

Genmodifierade djur på väg ut från labbet

Genmodifiering för nog mest tankarna till växter och grödor. Men även djur, bakterier och jäst kan genetiskt förändras. Ett exempel är genmanipulerade myggor i Brasilien.

May 2013
[Genetically modified animals on their way out of the lab]

Företagen flyr Sverige men GMO-forskningen fortsätter

PUBLICERAD: ONSDAG 5 JUNI 2013, 06:43 • UPPDATERAD: TORSDAG 12 SEPTEMBER 2013, 14:51

Motståndet mot GMO-grödor fick den sista kommersiella aktören att lämna Sverige. Inga nya odlingar planeras längre. Men frågan om GMO är långt ifrån ett avslutat kapitel. Just nu pågår flera forskningsförsök runt om i landet, bland annat i Umeå, Halland och Skåne. Och allt fler genmodifierade grödor importeras.

June 2013
[The companies are fleeing Sweden but GM research continues]

EU consumers less negative about GMOs than previously believed

Consumers in the EU do not seem to be much more skeptical of GMOs in food than consumers in other parts of the world, in contrast to what has previously been argued. The reason for this misconception is to a large extent that wording and the context of the questions in a survey can affect the answers.

In a meta-study combining the results from over 1,600 questions in 241 different studies in 58 regions a team of Mistra Biotech researchers shows that previous conclusions on the Europeans' negative attitude towards GM food might be the result of slightly different questions having been asked in Europe compared to other countries.

September 2013
Our meta study on attitudes to GMO among consumers attracted attention in both national and international media.

Minskat motstånd mot GMO

Trots att modern genteknik förändrat den globala matproduktionen i grunden har det europeiska motståndet varit kompakt. Nu höjs dock allt fler röster höjs för att lätta på de hårda restriktionerna - samtidigt som ny teknik utmanar gränserna för vad som räknas som genmodifierad mat.

Josefin Jakobsson, Peter Alestig
Publicerad 2014-08-16

August 2014
Erik Alexandersson and others were interviewd about their research on potato. [Reduced resistance to GMOs]

Want to link genes to complex traits? Start with more diversity

Date: September 18, 2014
Source: Genetics Society of America
Summary: Life is rarely simple. From crop yields to disease risks, the biological characteristics people care most about are considered 'complex traits,' making it hard to identify the genes involved. Standard methods for tracking down such genes usually only implicate a broad genomic region, and the identities of the crucial gene/s remain a mystery. Now, geneticists are embracing a powerful approach that pinpoints more precise areas of the genome.

September 2014

Professor: Köttfabrik för kloning en återvändsgränd

December 2015
Lotta Rydhmer in Vetenskapsradion (science broadcast on the Swedish public radio) Meat factory for cloning is a blind alley.

"Hundratals miljoner är undernärda - därför är genmodifierad mat en moralisk skyldighet"

December 2015
Payam Moula in Veckans Affärer. ["Hundreds of millions are malnourished - therefore genetically modified food is a moral obligation"]

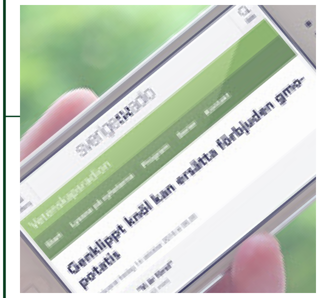
Genmodifierad potatis banar väg för miljövänlig och effektiv odling

August 2016
Erik Andreassson in Sydsvenska Dagbladet about environmentally friendly GM potato. [Genetically modified potatoes pave the way for environmentally friendly and efficient cultivation]

Går hög produktion och etik hand i hand?

I slutet av augusti höll Växa Sverige sin årliga konferens D & U, om djurhälsa och utfodring. Temat för dagarna var ökad produktion. Under en programpunkt var det ett panelsamtal på temat produktion och etik.

September 2016
Jordbruksaktuellt reported from a conference in which Helena Röcklinsberg was one of the speakers. [Do high production and ethics go hand in hand?]



October 2016
The Swedish public radio, among others, reported about Mariette Andersson's research on the use of CRISPR/Cas9 technology. [Gene-cut tuber can replace banned GMO potatoes]


JORDBRUKSAKTUELLT

MARKNAD Brödvete FOB Sverige **202 SEK** Diesel **11,14 SEK** Euro **10,48 SEK**

Potatisen förenar grannländer

Vid Sveriges lantbruksuniversitet (SLU) har hållbar potatisodling för framtiden undersökts. Utökat samarbete inom norden gällande växtförädling, mer pengar till forskning och större fältförsök är vad som skulle behövas inför framtiden.

Sverige, Norge och Finland är förenade genom potatisen. För det första har vi en förkärlek till mjölig potatis istället för fast som man föredrar i många andra länder. Sedan har vi ett gemensamt nordligt klimat; långa sommardagar och utmaningar kring samma sjukdomar. Torka är däremot vanligen inte något problem här.

Med detta som grund föreslår nu fyra forskare från SLU att de tre grannländerna borde börja samarbeta för att ta fram nya och bättre potatissorter. Detta arbete är nämligen

I tidskriften Potato Research publicerade nyligen forskare på SLU en analys av ett möjligt nordiskt samarbete kring hållbar potatisodling och förädling av

December 2016
[The potato unites neighbouring countries]

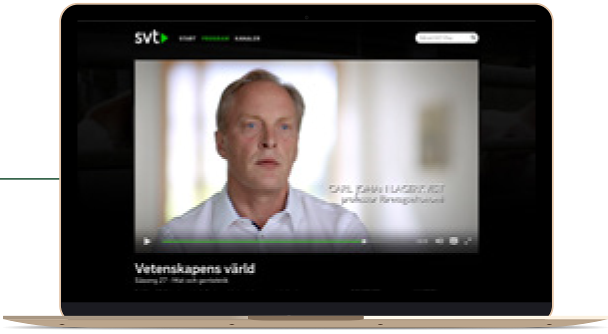
December 2016
[Genetically modified potatoes provide bioplastics]

› LANTBRUK 22 MARS 2017

GMO-växt tar upp mer organiskt kväve

"En milstolpe i vår forskning", säger professor Torgny Näsholm vid SLU i Umeå.

March 2017
[GMO plant takes up more organic nitrogen]



October 2017
Carl Johan Lagerkvist talked about consumer acceptance of gene technology in a science programme on Swedish television. [Vetenskapens Värld, Mat och genteknik]
Carl Johan and Lotta Rydhmer also participated in an on-line chat following the episode.


Genetic technologies rely on active roles for researchers, industry

December 2017

January 2018
Xue Zhao's study on dietary fiber in high amylose potato generated broad media attention. [Extra fibre in genetically altered potato]

Genmodifierad soja konkurrent till svenska proteingrödor

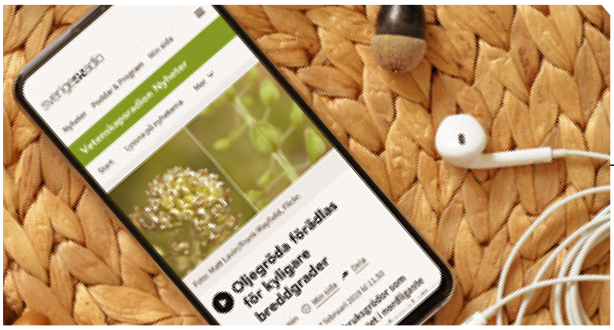
February 2018
A publication from 2017 that gained media attention was the study on potential effects if Sweden decides to import GM soy. [Genetically modified soy competes with Swedish protein crops]

Forskare har hittat 30 arvsanlag som ska ge ny oljeväxt bra egenskaper

June 2018
[Researchers have found 30 genes that will provide new oil crop with good traits]

Forskare: EU-beslutet hotar växtforskning

September 2018
[Researchers: The EU decision threatens plant research]



Oljegröda förädlas för kyligare breddgrader

July 2019
[Oil crop is bred for chilly latitudes]

Field cress tested as new protein source for pigs

September 2019

Konsumenterna är osäkra på vad märkningen "bioteknik" innebär. Foto: Anna Lehman, SLU

Konsumenter reagerar negativt på ordet "bioteknik"

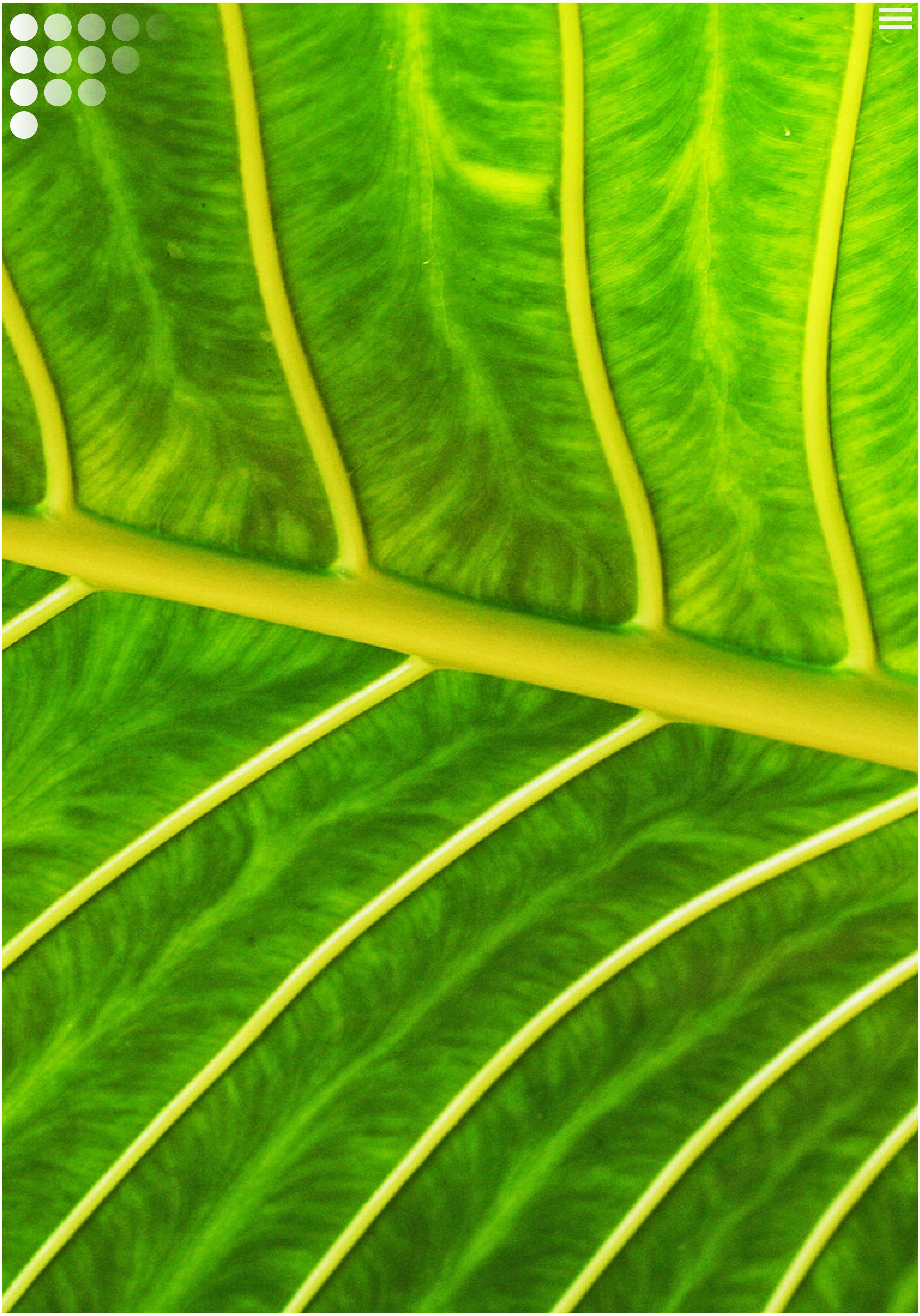
22 oktober, 2019

Märkning av livsmedel bör kombineras med information om vad orden i märkningstexten betyder. Det gäller inte minst termen "bioteknik".

October 2019
[Consumers react negatively to the word "biotechnology"]

2020
Dennis Eriksson interviewed in Lantbruksnytt about the regulations on genetically modified crops.

MISTRA BIOTECH 2012-2020



KICKED-OFF FROM MISTRA BIOTECH

Projects and collaborations

Mistra Biotech has been a breeding-ground not only for new crops and livestock but also for new research projects:

- SLU Grogrund has granted funding for 2019-2022 for breeding of starch potato. The research will be performed by Folke Sitbon, Mariette Andersson, Per Hofvander and Lyckeby Starch AB.
- Formas has funded a project for 2019-2021, aimed at defining breeding targets for potato starch quantity and granule size distribution and structure. The project will be performed by Mariette Andersson, Roger Andersson, Per Hofvander and Lyckeby Starch AB.
- Formas has granted a project for 2019-2021, aimed at enhanced utilization of an industrial side stream for a circular biobased economy, including superior nutritional protein. The research will be performed by Mariette Andersson, Carl Johan Lagerkvist, Mathias Eriksson, Per Hofvander, Folke Sitbon, Eva Johansson and Lyckeby Starch AB.
- The Mistra Biotech research in oat breeding will be continued in a collaboration with Nordic oat breeders (ArcticOat) that will start in 2020. Elisabeth Jonas and Catja Selga will take part in the network.
- The SLU participation in the 1000 bull genomes consortium (www.1000bullgenomes.com) was initiated through Mistra Biotech.
- The Swedish participation in the SUSAN ERA-NET project REDIVERSE, aimed at increased conservation and use of red dairy cattle breeds in Europe, was based on research performed in Mistra Biotech.
- Viking Genetics has funded a studentship under the LivID initiative, DNA-Tinder for cows.
- Klara Fischer is one of the researchers in the GEAP3 network *Genome Editing and Agricultural Policy, Practice and Public Perceptions* that is funded by trough Erasmus+.
- Dennis Eriksson worked nine months in Brussels at the European Plant Science Organisation (EPSO), with funding from the Mistra Fellow Programme.
- Dennis Eriksson has received funding from the European Cooperation in Science and Technology (COST) during 2019-2023 to develop networking activities and stimulate international collaborations in the field of plant genome editing.

NEW PLATFORM ON PLANT GENETIC RESOURCES

In the autumn 2018, the Plant Genetic Resources International Platform (PGRIP) was launched. The platform will facilitate interdisciplinary collaborations to address different policy and regulatory aspects for plant genetic resources in research and breeding. Resources are allocated to publications and young researcher mobility. The initiative is led by Dennis Eriksson and funded by Mistra and Mistra Biotech during 2018-2020.

www.pgrip.org

PGRIP

REPORT ON GENE EDITING TO THE SWEDISH BOARD OF AGRICULTURE

The programme has provided the Swedish Board of Agriculture (Jordbruksverket) with information about the consequences for Swedish research of the European Court's 2018 decision on gene edition technologies. In our answer, we used potato breeding as an example. Gene editing can be used to produce a potato with improved nutritional value. The same technology can be used to make potato resistant against major pests, thereby substantially reducing the need for pesticides in potato cultivation. Other modifications of potato, achievable with the same technology, can make it possible to produce in new plastic materials, which will be biologically degradable and will also have the advantage of replacing fossil oil. After more briefly mentioning other uses of gene editing, we concluded that important research goals, not least goals related to sustainability and climate adaptation, will be much more difficult to achieve if gene editing cannot be used as a breeding tool.

Letter from Mistra Biotech to the Swedish Board of Agriculture concerning the consequences for Swedish research of the European Court's decision (C-528/16) on new mutagenesis technologies, 3 December 2018

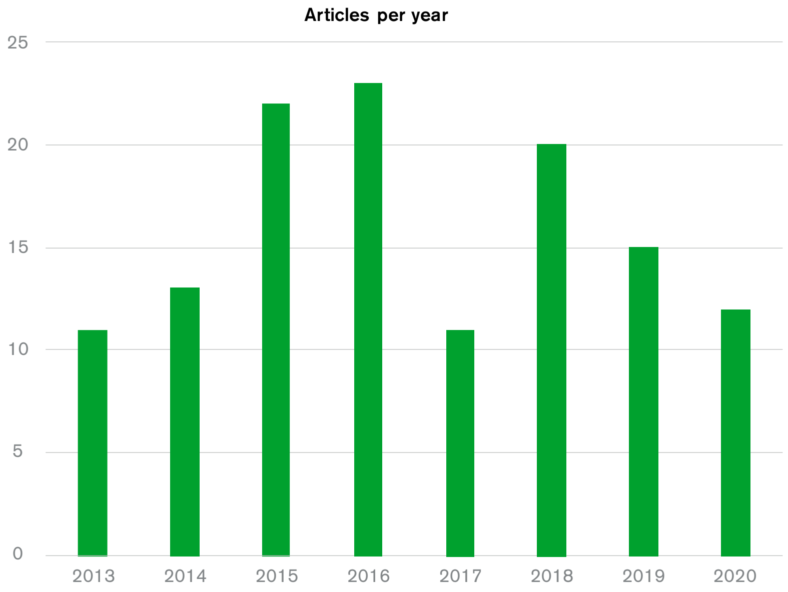
MOST COMMON SUBJECT CATEGORIES

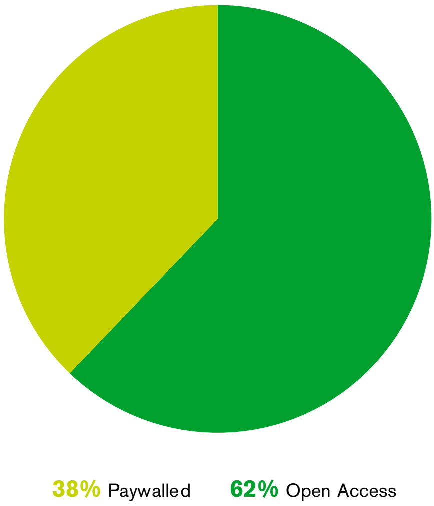
SEVERAL ARTICLES COUNTS IN MULTIPLE CATEGORIES

Bibliometrics

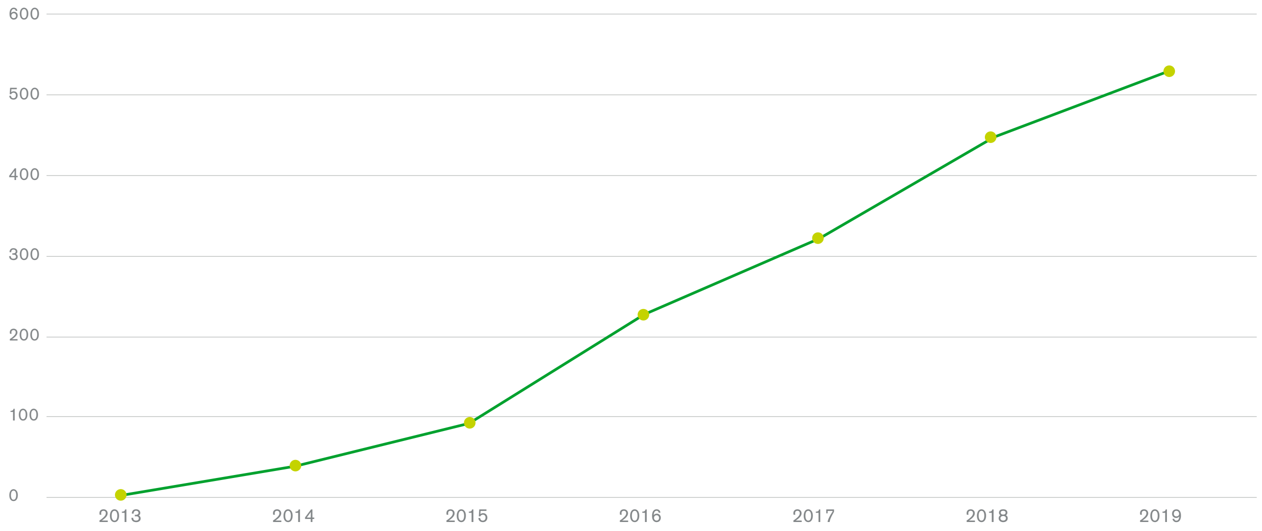
The graphs below are based on the publications that were listed in Web of Knowledge in May 2020. Note that many articles are recently published or on their way to publication, and consequently not included in the statistics.

ARTICLES PUBLISHED IN SCIENTIFIC JOURNALS





CITATIONS PER YEAR (1889 IN TOTAL, AVERAGE 18 PER ARTICLE)



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The total budget for phase 1 (January 2012- March 2016) was 80.2 MSEK, of which 40 MSEK was Mistra's funding, 40 MSEK SLU's co-funding, and a 0.2 MSEK a contribution from Lantmännen. In kind contributions were made by Lyckeby Starch AB and the partner universities. In phase 2 (April 2016-March 2020), Mistra contributed 54 MSEK and SLU co-funding with 50 MSEK, half of which (25 MSEK) took the form of in kind departmental co-funding. In 2019 Mistra assigned 0.827 MSEK to an international, multidisciplinary network for the governance of plant genetic resources, led by Dennis Eriksson and overseen by the Mistra Biotech Board.

Abbreviations and scientific terms

Accession	Plant material from a species which is collected at one time from a specific location.
Allele	Alternative form of a gene.
Amorphous	Of indeterminate or irregular form, with no visible differentiation in structure.
Biennial	About a plant: that lives for two years.
Breeding	Production of offspring with particular characters, applied in animal husbandry, agriculture and horticulture to improve desirable qualities.
Chromatography	Separation of compounds from a mixture on the basis of their affinity for migration with a nonpolar solvent.
Chromosome	Structure of DNA and associated proteins.
Cloning	Production of a group of genetically identical individuals, cells or DNA molecules.
CRISPR	Clustered regularly interspaced short palindromic repeat. Part in the cellular machinery that finds the matching nucleotide sequence in the genome. Used in site-directed mutagenesis, also called genome editing.
Cultivar	Plant variety that is not normally found in wild populations.
Cytogenetic	Combining cytology (the study of the structure, function and life history of the cell) with genetics.
DNA	Deoxyribonucleic acid. The large molecule that stores the genetic information in all cells.
Domestication	The selective breeding by humans of plant and animal species in order to accommodate human needs.
Effector	Pathogen molecule that manipulates the immune system in plants.
Elastic Net regression	Statistical method used to estimate the relationship between different variables.
EFSA	European Food Safety Authority.
Endometrial epithelial cells	Cells lining the inner surface of the uterus.
Florescence	The act, state or period of flowering.
GEBV	Genomic breeding value.
Gene	The basic unit of inheritance, by which hereditary characteristics are transmitted from parent to offspring.
Gene editing	See genome editing.
Genetic engineering	Any change in the genetic constitution of a living organism that has been brought about by artificial means and which usually would not occur in nature.
Genome	The complete set of genes carried by an organism.
Genome editing	Method to cut the DNA at a specific site. It may or may not include insertion of DNA. Also called gene editing or site-directed mutagenesis.
Genomics	Interdisciplinary field focusing on the structure, function, evolution, mapping, and editing of genomes.
Genomic selection (GS)	Breeding method that makes use of genomic information from an individual to estimate its breeding value, without knowing the positions of specific genes.

Genotype	The genetic constitution of an organism.
GI	Glycaemic index, a measure of how fast the body breaks down carbohydrates to glucose.
GM	Genetic modification/genetically modified.
GMO	Genetically modified organism.
GWAS	Genome-wide association study, a study of correlations between a large number of genetic markers and specific traits in an organism.
Heritability	Proportion of the variation in a trait in a population that depends on genetic variation.
Heterozygosity	The presence of different variants of a gene on homologous chromosomes (the two chromosomes that form a pair) in an individual.
Hubris argument	The argument that some activity goes beyond what we humans have a right to do.
Hybridization	Formation of a cross-bred animal or plant.
Inbreeding	Production of offspring from the mating of individuals that are closely related genetically.
In vitro	Performed outside the organism from which the material is derived.
Locus	Specific place on a chromosome where a gene is located.
Marker	Gene or other DNA of known location used for identification purposes.
Mass spectrometry	Technique for the measurement of atomic and molecular masses.
Metabolite	An intermediate product or end product of the life-sustaining chemical reactions in an organism.
Microsatellite	Region of repetitive DNA in which certain DNA sequences are repeated, typically 5–50 times.
Mutagenesis	A process by which the genetic information of an organism is changed, resulting in a mutation.
Mutation	A change in the sequence of nucleotides in the genome of an organism.
Mycorrhiza	An association between a fungus and the roots of a plant, from which both appear to benefit.
NGO	Non-governmental organization.
Nuclease	Enzyme that cleaves the bonds between nucleotides.
Nucleotide	The basic subunit of DNA (adenine, thymine, cytosine, and guanine) and RNA (adenine, uracil, cytosine, and guanine).
Oligonucleotide	Short, single-stranded DNA or RNA molecule.
Outcrossing	Crossing of plants or animals that are not closely related genetically.
Pedigree	The ancestral history of a group of related individuals.
Peptide	Chain of a small number (up to around 20) of amino acids held together by peptide bonds.

Perenniality	About a plant: living more than two years.
Phenotype	The combination of all observable characteristics of an organism (in contrast to the genotype, its genetic characteristic).
Pod shattering	The dispersal of a crop's seeds when they become ripe.
Probit regression	Statistical model for the relationship between a dependent and an independent variable, where the dependent variable can take only two values
Proteomics	The large-scale study of proteins.
QTL	Quantitative trait locus, DNA sequence containing or linked to a gene coding for a quantitative trait.
RNA	Ribonucleic acid, a family of molecules that perform coding, decoding, regulation, and expression of genes.
RNAi	RNA interference, method to down-regulate the expression levels of target genes.
SDM	See site-directed mutagenesis.
Selfing	Self-fertilization, the union of male and female gametes from the same individual.
Seminal plasma	The complex fluid medium that spermatozoa are suspended in.
Site-directed mutagenesis	Method to cut the DNA at a specific site. It may or may not include insertion of DNA. Also called gene editing or genome editing.
SNP	Single-nucleotide polymorphism, single bases in the genome that vary between individuals in the population.
SP	Seminal plasma.
Spermatid	Cell in the testis which develops into a sperm. Contrary to other cells in the body, the spermatid and the sperm contain only one copy of each chromosome.
Spermatocyte	Cell in the testis from which spermatids are formed.
Spermatozoon	Mature motile male gamete in animals.
SRB	Swedish red white cattle.
TALEN	Transcription activator-like effector nuclease, a type of site directed-mutagenesis.
Transformation	Genetic alteration of a cell resulting from uptake and incorporation of DNA from its surroundings.
Transgenesis	The process of introducing a gene from one organism into the genome of another organism.
Transgenic	About an organism: containing genes from another species that have been deliberately introduced though genetic modification.
Vernalization	Exposure of plants or their seeds to a period of cold to induce the flowering process.