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Nistra Biotech Annual Report 2018

MISTRA Biotech

Mistra Biotech

Annual Report 2018

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MAIN FUNDERS



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.

"Promising projects that could improve the nutritional value of foodstuffs will also be blocked, unless the European regulations are changed."

– Inger Andersson

CHAIR'S PREFACE

High time for a modern legislation

In July 2018 the European Court made a decision that is predicted to have a large impact on plant breeding in Europe. The court determined that gene editing with methods such as CRISPR/Cas9 will be subject to the same rules and restrictions as traditional genetic modification (GM). However, since the ruling concerns a specific legal case, its general implications are not entirely clear. The Swedish Board of Agriculture has asked the research community for information and comments on the consequences of the ruling. In December 2018, Mistra Biotech sent in an extensive answer that is based on information from researchers in the programme.

It seems unavoidable that the ruling will prevent the use of cultivars obtained with the new technologies on European farmlands. This is because the approval process for GM cultivars is so expensive, protracted, and politically restrained, that it is more than ten years since a crop variety classified as genetically modified was licensed for cultivation in Europe.

In our answer to the Board of Agriculture we emphasized that it will now be even more challenging for Swedish farmers to adapt to climate change. Since one of the most important breeding tools cannot be used, it will take longer time to obtain the new cultivars that farmers will need to cope with a changing climate, and to reduce the impact of agriculture itself on the climate.

The ruling also blocks important opportunities to reduce the use of pesticides. Researchers in Mistra Biotech have obtained promising results in resistance breeding of potato with the CRISPR/Cas9 technology. Due to the court's decision, the new, resistant potato cultivars are unlikely to be used in practice. This is unfortunate since the rather extensive spraying of potato against late blight will continue. The same applies for instance to controlling leaf blotch in barley.

Other researchers in Mistra Biotech have bred a more healthy potato ("amylose potato"). The results are very positive, but since CRISPR/Cas9 was used to obtain them, this product has very slim chances of reaching consumers. (If by chance, a potato plant was found that had obtained the same genetic changes through spontaneous mutation, then it could be grown commercially.) Other promising projects that could improve the nutritional value of foodstuffs will also be blocked, unless the European regulations are changed.

It now becomes increasingly difficult to keep up world-class plant breeding in Sweden. Breeders need co-operations with farmers who can test their research outcomes in practice, and also with a breeding industry that can produce the new cultivars on a large scale and make them available to farmers. We have already seen movements of breeding industry from Europe to countries with a more modern legislation.

From a legal point of view, it is worrisome that we now seem to have a legislation whose application cannot be controlled or enforced. Neither DNA analysis nor any other analytical method can inform us whether a plant has been obtained with the new gene editing techniques or in some other way, such as spontaneous mutation or radiation-induced mutation (a conventional method not affected by the decision). This cannot be solved by developing new analytical methods. A plant can have obtained exactly same change in its DNA either with CRISPR/Cas9, with radiation treatment, or as the result of spontaneous mutations. Not only the DNA is the same in both cases, there are also no other differences in terms of traits or chemical composition.

This can cause considerable problems if a plant breeder wants to import and use a cultivar from a country where the new technologies can be used freely, without any demands of documentation. It is impossible to determine if the plant was obtained with gene editing technologies or in some other way. How can legislation be upheld if there is no way to determine whether it is complied with? We may also ask what the use can be of such legislation. The properties of the plant, and the food produced from them, will be the same independently of how it was obtained.

We have reasons to worry about the long-term effects of this legislation on agriculture, both in Sweden and the rest of Europe. The new technologies will be used in other parts of the world. Farming in Europe is at risk of becoming less and less competitive. The European Commission has many other important issues to deal with, but a thorough modernization of the legislation on plant breeding cannot wait any longer.

Inger Andersson

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





"When a mere possibility argument is promoted one-sidedly and aggressively, a wrongful impression can be created that there must be some substantial argument behind it."

– Sven Ove Hansson

How to be cautious

The precautionary principle has often been invoked in debates on modern biotechnology. Opponents claim that plants obtained with gene editing or genetic modification should not be used, due to the possibility that they might have negative effects on human health and the environment.

This sounds convincing. But does the argument hold? Let us have a closer look at the precautionary principle. Contrary to what many seem to believe, it is not just a general appeal to be cautious. It is a rather specific principle for the evaluation of evidence, originating in international discussions on environmental policies in the 1980s. It is now incorporated in European legislation and several international environmental treaties. These documents leave no doubt that the principle refers to scientifically reasonable suspicions of danger, not to suspicions that have no support in science.

For an example of when the principle can be applied, suppose scientists have shown that animals exposed to high doses of some chemical have an increased incidence of cancer. This makes it plausible but not certain that humans who are exposed to lower doses of the same substance also run an increased risk of cancer. The precautionary principle tells us that in such cases, it is justified to take precautionary measures in order to minimize human exposures.

What about genetically modified crops? Here the situation is different. There is a strong and wellfounded scientific consensus that the fact that a plant has been obtained with genome editing or gene modification is no reason to believe or suspect that it is dangerous to human health or the environment. Statements issued by a large number of academies and other scientific organizations confirm that this consensus is as strong as that on anthropogenic climate change. (In both cases, beware of vociferous "dissenting experts" with no standing in the relevant expert communities.) Therefore, the (science-based) precautionary principle gives us no reason to refrain from using genome edited or genetically modified plants.

But, some would say, we cannot be sure that the scientists are right. Isn't it perfectly possible that genetically modified plants are dangerous in some way that scientists have not yet discovered? And isn't this possibility reason enough to refrain from using these plants?

This is a *mere possibility argument*, an argument saying that we should worry about something just because it could possibly happen. The problem with such arguments is that they can be constructed for and against almost anything. For instance, think of some foodstuff that you eat. It is (merely) possible that it has some serious long-term health effects that scientists have not yet discovered. But that is no reason to stop eating it, since the same applies to anything that we eat.

When a mere possibility argument is promoted one-sidedly and aggressively, a wrongful impression can be created that there must be some substantial argument behind it. But relying on such impressions can lead to disastrous decisions. For instance, this is how many parents have been misled to refrain from vaccinating their children against deadly diseases such as measles.

Whenever there are reasonable, science-based suspicions that a crop or variety can have adverse health effects or cause environments problems, then we should apply the precautionary principle, and take the necessary measures to prevent harm. And we should do this irrespectively of what technologies the plant breeder has used. But we should not do so on the basis of suppositions that have no support in our best knowledge of the world.

There *might* be a wolf outside of my window just now. I do not know, since I have not looked out for a while. But that is no reason to cry wolf!

Sven Ove Hansson

Programme Director, Professor in Philosophy at the Royal Institute of Technology (KTH)





PRODUCTION SYSTEMS WITH FIELD CRESS

COMMUNICATION

WITH GENETIC MODIFICATION

TRADE REGULATIONS & GMO PRODUCTS

PUBLIC DISCOURSE ON BIOTECHNOLOGY

RA2 REFINED TOOLS FOR

MOLECULAR BREEDING

PUBLIC OPINION & GMO FOOD REGULATIONS

PRODUCT-BASED REGULATORY SYSTEM FOR GENETICALLY MODIFIED VARIETIES

RA3 SYNTHESIS & SOCIAL ANALYSIS

Mistra Biotech

Mistra Biotech is an interdisciplinary research programme focusing on the use of biotechnology for sustainable and competitive agriculture and food systems. Our vision is to contribute to the processes that will enable the Swedish agricultural and food sector to produce an increased amount of high-quality, healthy food at moderate costs with less input, decreased environmental impacts, and healthier crops and livestock. The goal is sustainable production systems from ecological, social, and economic perspectives. We perform research in both the natural and the social sciences.

Our research in the natural sciences is aimed at utilizing the potential of agricultural biotechnology to contribute to a more sustainable food production with healthier products and reduced environmental impacts. With ability comes responsibility, and we take the concerns that have been raised about potential negative effects of biotechnological products on human health and the environment very seriously. For us, safety, control, and transparency are essential regardless of which technology is used.

Our research in the social sciences involves social, economic, and ethical aspects of the use of biotechnology in agricultural production, with a strong focus on sustainability issues and on the perspectives of stakeholders in the food production systems. The first phase of the programme started in 2012. During 2016 the programme entered its second phase (2016-2020) and reorganized its research in order to put more emphasis on programme synthesis and policy issues. Mistra Biotech now consists of three research areas (RA): RA1 Development of innovative plant products using modern breeding tools, RA2 Refined tools for molecular breeding, and RA3 Synthesis and social analysis. Mistra Biotech involves about 60 researchers. Most are at SLU, but some work at KTH, Lund University, Roskilde University and Uppsala University. The programme also includes collaborations with the University of Copenhagen, the University of Edinburgh, and other institutions. Phase 1 (2012 to 2016) was funded by Mistra with 10 million SEK per year and co-funded by SLU with the same amount. Lantmännen also contributed financially with a sum of 50,000 SEK per year during the first phase. In phase 2 (2016-2020) Mistra and SLU continue their support, with additional funding from Lantmännen (800,000 SEK), Graminor (770,000 SEK), and Lyckeby Starch AB (200,000 SEK) for the remaining four years. Many companies, agencies, and organisations also support the programme with their knowledge and advice.

We use the term "biotechnology" in a broad sense that includes (but is not limited to) the use of genomic tools, molecular markers, genetic modification, and genome editing as well as technologies for cell and tissue culture and for animal cloning.





RA1 DEVELOPMENT OF INNOVATIVE PLANT PRODUCTS USING MODERN BREEDING TOOLS

The major focus in this research area is the breeding for late blight resistance and altered starch composition in potato, and the development of field cress (Lepidium campestre) into a new oil and cover crop with several improved agronomic traits. The genetic improvement of target traits is carried out by using conventional breeding, genetic modification (GM), site-directed mutation including the new CRISPR/Cas9 technology and other non-GM approaches. We also analyse the characteristics and health aspects of the potato starch and the field cress seed oil of improved lines. Apart from the assumed reduced nutrient leaching through the domestication of the biennial, and potentially perennial, catch crop field cress, we address this issue through increasing plant nitrogen use efficiency in potato. We also work with improving leaf blotch resistance in barley.

<u>RA2</u>

REFINED TOOLS FOR MOLECULAR BREEDING

In this research area the central focus is the improved use of molecular information in crops and livestock breeding by refining the tools for genomic and proteomic selection. Based on prior information on genetic variation and mathematical models of resource allocation we can differentiate among genomic regions in the selection process to improve feed efficiency in livestock. We will evaluate scenarios for genomic selection in crossbreeding in the context of current and potential future scenarios in livestock. We develop new diploid potato clones, implement genomic selection in existing potato breeding material, and investigate new ways to select for improved resistance against F. graminearum in oats. In our work on proteomics we search for peptides to be used as markers in potato breeding together with genomic information. The same approach is used in the work on bull fertility where we use our previously gathered information on a larger cohort of bulls.

RA3 SYNTHESIS AND SOCIAL ANALYSIS

It is not sufficient to produce new crop and livestock varieties and breeds, with all the desirable properties. The new products also have to be introduced to, and accepted by the farmers, the food industry and the consumers. This raises a wide range of issues: environmental effects, economic viability, legislation, attitudes and preferences among consumers and other stakeholders, as well as ethical considerations. This research area is devoted to analyses of these factors.

We perform field trials with the plants developed in RA1 in order to provide knowledge about agricultural properties and ecological consequences. The field trials also provide seeds and tubers for analyses of oil and starch quality (RA1), phenotypes for genomic analyses (RA2), and opportunities to communication activities that we use in studies of consumer attitudes.

Several studies focus on the regulatory system in the EU and its effects on the use of biotechnology to make agriculture and food production more sustainable from an environmental, economic, and social point of view. We highlight ethical argumentation for and against different designs of the legislation. We use a hypothetical market introduction of genetically modified field cress as a case study, investigating scientific, regulatory, economic, and ethical barriers to its introduction, and arguments concerning naturalness, precaution, fairness, labelling, and consumer autonomy. We also perform a case study of the use of GM feed for animals in Swedish meat and dairy production, including a value chain analysis estimating the costs of segregation.

In a simulation study that includes genetic and economic investigations, as well as an ethical analysis, we analyse breeding programmes for GM livestock for food production.

We also investigate how consumer attitudes to breeding biotechnologies are influenced by different types of information, and analyse farmers' perspectives on the use of such technologies.





"I wish that more food companies took responsibility for sustainable development on this issue by not confirming the consumers' irrational fear."

– Sara Sundquist

Sustainable food production needs modern biotechnology

The Steven Spielberg movie Jaws from 1975 has become one of film history's most famous horror movies. The plot is a classic; a seven meter long white shark brutally attacks and devours people. The movie made people scared of swimming in the ocean and has created a horror of the white shark that still makes us perceive it as the bloodiest animal on the planet, even though it kills fewer people than both alligators and wild cat species. And the media consistently report more frequently on shark attacks than on attacks of other animals.

About ten years before *Jaws*, in 1962, the movie *The day of the triffids* was launched. It is based on the book with the same name where invasive, carnivorous plants from space are about to eradicate mankind. The lead researcher initially believes that the invasive plants are genetically modified plants developed by the Soviet Union. In the 80s it was re-launched as a TV series.

I sometimes think that *The day of the triffids* has had a greater influence than we might think on the phobic view on genetic modification of crops that can be found among some consumers. In the same way as a movie like *Jaws* obviously influenced our ability to judge the danger of sharks, a film about modified plants that spread spontaneously might have created a similar concern for a "what if" scenario with regard to plants, although they are seen as the sweetest, most innocent and important part of nature.

Researchers are trying to understand why the gap between science and the perception of genetic modification is so large. Not in any other area can an equal discrepancy between science and the public be found. The scientific community agrees that the genetic modification of plants is as safe as other plant breeding methods, but more than 50 percent of the consumers are skeptical, often with uncertainty as justification. The problem is that this perception contributes to a legally locked position where the regulatory framework is expected to respond to public concern and assure them of high health requirements. The first European law on genetically modified organisms came already in 1991 and was based on the knowledge and paradigm of that time. In the almost 30 years that have passed since then the development in biotechnology has way passed the legislation, which became painfully clear in July 2018*. It is however not the first time in history where the research has outdated the legislation affecting it.

We do need modern plant breeding. For a long time, the discussion from a food industrial point of view has had its focus on economic aspects. Today, those motives are secondary. We need a functioning and applied crop development for the climate and for the environment as a whole. Even if we manage to keep global warming well below two degrees, the stresses on the agricultural systems will be huge, and it is not a wild guess that some of the crop varieties we use today will have to be replaced or adapted in order to cope with the changed conditions. Given the rapid pace of climate change, we do not have time to wait the 15-20 years that it normally takes to develop a new crop variety with the desired traits, and sometimes it might not even be possible with conventional methods. If we are to succeed in the transition, we need to be quick, and then it is directly counter-productive to counteract a technology which may enable agriculture to provide us with food despite population growth and changes in the agricultural landscape.

If we facilitate the breeding we can produce varieties with other desirable traits, crops with benefits for both the human health and the environment.

The time has come to deal with the fear of the triffids. I wish that more food companies took responsibility for sustainable development on this issue by not confirming the consumers' irrational fear. We need to challenge the misconceptions about modern plant breeding, not fuel them.

Sara Sundquist

Sustainability Manager and Industrial Policy Expert at The Swedish Food Federation

*The European Court of Justice ruled that plants that have been genome edited (for example by using CRISPR/Cas9) should be regulated as GMOs.













Mistra Biotech selections from 2018

In the following section we present research highlights during 2018





Breeding of field cress

The domestication of field cress (*Lepidium campestre*) into a new oil and catch crop is progressing, and the plant might even be considered a crop rather than a wild plant in a near future. The researchers are continuing to improve different traits, and combine key traits into the same breeding lines. The field trials have shown promising results in 2018, despite the very dry summer.

STACKING OF IMPROVED TRAITS

During 2018 Li-Hua Zhu and colleagues have combined (stacked) important traits, previously achieved through genetic transformation, into single breeding lines: Lines with high oleic acid content and improved pod shatter lines were crossed as well as high oleic acid lines and wax ester lines. The second generation hybrids had the same level of wax esters as the original parental line (30 percent of total oil content, the wild type has zero percent) and a higher level of oleic acid compared to the wild type. Stacking of genes is also made to increase the total oil content of the seeds, in this case it is the plant hemoglobin and *WRI1* genes that are combined.

FIELD TRIALS WITH LEPIDIUM HYBRIDS

In parallel to improving traits by genetic transformation and genome editing, Mulatu Geleta and colleagues are improving the field cress through crossing selected field cress breeding lines with other *Lepidium* species. From 176 previously evaluated hybrid lines, 44 have been selected to evaluate their performance as under-sown in spring barley. Those trials are continuing. The selection was mainly based on germination, growth vigour, weed competiveness and flowering time (earliness), pod shatter resistance and seed yield.

From the same 176 lines, the researchers also selected eight early maturing lines and 16 late maturing lines for field trials at different locations in Sweden. After the season 2016/2017 the researchers made seed germination tests and changed the planting technique at the field station Lönnstorp (in the south of Sweden). This resulted in a better establishment of plants in the field and almost doubled the seed yield (nearly six tons per hectare) despite the dry summer of 2018. At the Umeå research station (in the north of Sweden) the old planting method (same as for oilseed rape) was still used which resulted in only up to 2.6 tons per hectare due to low plant establishment in the field. The 24 breeding lines have been planted again for the 2018/2019 crop season, both as under-sown in barley and as sole crop at Lönnstorp, Umeå and Lanna (outside Lidköping).

The first batches of interesting tri-species (crossings of *L. campestre*, *L. heterophyllum*, and *L. hirtum*) second generation hybrids were harvested in 2018. Seeds from the best plants are now included in a new field trial at Lönnstorp along with perennial breeding lines (compared to the regular biannual lines).

MOLECULAR MARKERS

Recently, great efforts have been made to speed-up the domestication process through developing genomics based breeding techniques. Mulatu Geleta and Cecilia Gustafsson have generated a first draft of the whole genome sequence of field cress. Its genome size is determined to be about 533 million base pairs, which is around four times the size of the *Arabidopsis thaliana* genome. In addition, 24 homologues of *Arabidopsis* genes, regulating various desirable traits, have been located on the field cress chromosomes. At the moment the researchers are studying the effects of the variation in these genes on the target traits in order to develop DNA markers that can be used in marker assisted selection in future breeding.

GENOME EDITING

Some traits are difficult to improve by conventional crossbreeding, either because they cannot be found in the available gene pool or because the coding region is tightly linked to less desirable traits. Previously the main tools for overcoming those hurdles have been mutagenesis (through physical or chemical mutagens), or genetic transformation (inserting DNA).

With the genome editing method CRISPR/Cas9 it is possible to target specific sites in the DNA and generate point mutations, without leaving any external DNA in the genome. The most efficient way to do this is to introduce the CRISPR/Cas9 vector (the protein/ RNA complex that finds and cut the DNA strand) into protoplasts (cells that had their cell walls removed). However, an efficient protocol for protoplast growth and regeneration needs to be developed specifically for this plant species. There are several crucial steps in this process and so far Li-Hua Zhu and colleagues have optimized the isolation and growth steps. They are now improving the regeneration and transfection steps.

In parallel the researchers are using the CRISPR/ Cas9 vectors to obtain field cress mutant lines through transformation. With this method the DNA coding





Field cress protoplasts. 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", regrow the cell wall, and then make the cell duplicate and grow into plant tissue.

for the CRISPR/Cas9 complex is inserted into the genome. After the CRISPR/Cas9 complex has generated the desired point mutations it is possible to get rid of the complex-coding DNA by self-crossing, resulting in mutant lines without the extra DNA insertion. The targeted field cress genes are involved in the transport of glucosinolates (GSLs) to the seeds. High levels of GLSs are unhealthy and therefore need to be reduced before the oil or the seed cake can be

used in food or feed. Because the levels of GSLs in the rest of the plant will not be affected, the GSLs' normal functions in plant defence against insects and pathogens will be maintained.

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When the field cress seeds are soaked in water a gel is formed around each seed. The gel, or seed gum, consists of high levels of pectin which is a common ingredient in food products.

Oil, seed cake – and pectin?

The original intention at the start of the domestication of field cress was to develop a new oil crop for Sweden. After the oil is being extracted from the seeds, the residual is left in form of a seed cake which can be used in feed. However, it turned out that when the seeds are soaked in water a gel is formed around the seeds. A seed gum. Several other seeds form gels in the same way, for example in chia (*Salvia hispanica* L.), garden cress (L. *sativum*), and linseed/flax (*Linum usitatissimum* L.).

But what does the gum contain? The gel was extracted in a common food mixer and the initial analyses show that the gel consists of up to 80 percent dietary fibre, and constitutes seven percent of the seed weight. Moreover there was a high proportion of uronic acid, galactose and rhamnose – a substance, better known as pectin.

Pectin is a complex of non-starch polysaccharides that is common in vegetables and fruits, especially in the outer layer of for example apples and in different citrus species. Pectin is a common ingredient in food products, where it is used for gelling or thickening or as dietary fiber enrichment. Dietary fiber binds to the cholesterol and bile acid in the gut and promotes their excretion, which results in lower cholesterol levels in the blood.

Pectin can also be used in gluten free bread as a thickener and stabilizer, something that was tested in the lab with positive results. The initial studies in the master project only included one breeding line of field cress and more lines need to be analysed to determine the variation in chemical composition. The physical behaviour also needs to be thoroughly examined to find out what kind of food application this pectin could be suitable for.

The tests were part of a master thesis by Anna Mannerteg and is published with the title "A pectic polysaccharide in seed gum of *Lepidium campestre*".

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Phenotypic (top) and genotypic (bottom) data from eight potato families and their two parents. The phenotype is all characteristics that you can measure or observe. The genotype is the genetic setup in each individual. By using genotypic data it is possible to select the right individuals at an early stage in development and without the interference of environmental factors.

Speeding up the selection in potato breeding

To be able to select the best plants for further breeding, molecular markers offer off-season selections at an early stage in the plant development. This saves both time and resources. Currently, the markerbased breeding in potato is limited to cover only a few genes affecting resistance to late blight, nematodes and viruses.

Catja Selga and Rodomiro Ortiz are combining marker-based selection with genomic selection for a number of both simple and complex traits. They have now identified sites in the DNA related to traits such as maturity, disease resistance and tuber yield.

The genomic data required for these methods are expensive to generate. Hence, the researchers have developed a bioinformatic "pipeline" that can reduce the costs for genotypic data, without losing information vital for the methods. This pipeline strategy includes a number of bioinformatical steps to reduce the number of genotypic markers. It will be published during 2019 and should give more breeders the opportunity to implement genomics-led breeding in their potato breeding programmes.

In SLU's breeding programme for potatoes, approximately 35 crosses are made every year. The programme aims for 10 000 new breeding clones in the first cycle of selection. Selections in the first generation are based on tuber characteristics (skin finish and colour plus size and shape uniformity). Tuber quality traits, such as cooking and frying quality, flesh colour, specific gravity and resistance against Phytophthora infestans (causing late blight in both foliage and tubers) are more important in the later cycles of selection. The researchers found the first cycle of selection to be the most time consuming, thus, introducing genomic selection at this step would have the largest impact. Currently, the researchers are testing the accuracy of their developed genomic selection method, using material from the SLU potato breeding programme. During 2019, the data will be further analysed before the method can be implemented in the breeding programme for Swedish potatoes.

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Genome editing for healthy potatoes

Mariette Andersson and her colleagues have found a new way of using the so-called genetic scissor CRISPR/Cas9 so that the DNA coding for the protein complex does not end up in the genome of the edited potato plant.

The researchers took cells from the potato, removed the cell walls and added the CRISPR/Cas9 complex (*i.e.* the gene scissor itself and not the DNA that encodes it). Then the cell cultures were grown into plants. The researchers checked that the mutations were in the right places, and examined whether any DNA was accidentally inserted into the genome. With this new "DNA-free" method, the researchers produced several potato clones with mutations in all four alleles of the gene (as most cultivated potatoes this was a tetraploid, thus having four sets of chromosomes) without any added DNA.

The gene the researchers chose to edit in the potato is involved in the biosynthesis of the amylose starch molecule. The gene got a mutation that prevented the production of amylose in the plant, and instead more of the amylopectin starch molecule was produced. Now the researchers are trying to do the reverse, *i.e.* to prevent the production of amylopectin in order to get more amylose, using the same new protocol. A high content of amylose gives a high content of resistant starch which is a dietary fiber with many health benefits. To be successful and get viable plants the researchers have to knock out the right number of alleles, so the activity of the enzyme affecting the branching of the starch molecule is lowered, but not completely shut down. Currently twenty potato clones with different combinations of silenced alleles are growing in the greenhouse in Alnarp.

The results on the successful genome editing without DNA insertion is published in the journal *Physiologica Plantarum* with the title "Genome editing in potato via CRISPR-Cas9 ribonucleoprotein delivery".

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Starch consists of amylose and amylopectin. Amylose has a low glycemic index and is therefore regarded as being healthier. The researchers have previously developed high amylose potatoes with genetic transformation. Now they are doing the same thing but without the addition of any new DNA.





Some potato varieties are better than others in defending themselves against Phytophthora infestans (that causes potato late blight). The researchers are trying to find out which proteins are involved in this defence.

Proteins used in the defence against potato late blight

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 whom the attacker is, and they have evolved during the evolutionary arms race between the plant species and the pest.

Svante Resjö, Muhammad Awais Zahid and their colleagues have performed an analysis to find out more about which plant proteins are involved in what kinds of plant defense systems, during the attack of potato late blight. They have looked at the composition and levels of specific proteins in leaves of potato, when the plant protects itself from the oomycete pathogen *Phytophthora infestans* (that causes potato late blight). For instance, a protein annotated as a sterol carrier protein shows high abundance in plants that are under attack, which is interesting, and in a way logical, since the oomycete relies on the plant host for sterols.

In addition to the sterol carrier protein, they found an increased abundance of several RNA binding proteins as part of the defence response. This kind of proteins have a role in a great number of processes in the cells 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. Plants 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 defense 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 by that initiate a second level of defense called effectortriggered immunity (ETI).

The researchers found that some changes of protein abundance are only regulated in PTI, and not at all in ETI interactions. One such potato protein has a domain resembling a part of a protein found in barley. And 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 reduction of jasmonic acid accumulation, indicating that an increase of this protein might contribute to a generation of signaling molecules needed for the PTI response. Jasmonic acid is a hormone well known for its function in regulating plant responses to stress.

Another interaction-specific change that the researchers noticed was that a family of catalase proteins only were 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.

The study is to be published and the results will hopefully be used in future potato pre-breeding to predict sustainable combinations of resistance genes in the plant.

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Biotechnology advocates apparently would like to see regulatory force to be uniformly relaxed, while biotechnology

skeptics desire the opposite move."

The definition of technology neutrality

Technology neutrality is sometimes thought to be a desirable feature of the regulations surrounding agricultural biotechnology. But what is it? The basic idea is that different technologies should be treated fairly.

Per Sandin, Karin Edvardsson Björnberg and their colleague Christian Munthe (University of Gothenburg) argue that technology neutrality must be understood in relation to the regulatory rationale, that is, the purpose of the regulation. They offer a definition of technology neutrality stating in effect that similar rules apply to technologies that are alike in terms of the regulatory rationale. 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 also has to be understood in scalar terms; a structure can be *more* or *less* technology neutral. Regulation may be viewed as quite technology neutral in relation to a specific regulatory rationale (such as safety) and at the same time viewed as quite technology specific with regard to some other regulatory rationale, for instance environmental impact.

The current legislation on genetically modified organisms (GMOs) 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.

A number of authors have previously argued that the current EU GMO regulatory processes are unfair and should be made more technology neutral. A typical statement is that similar cases should be treated uniformly and that differences in treatment must be justified.

Requirements of coherence in the EU GMO legislation are used as a basis for requiring that similar regulatory arrangements should apply to relevantly similar technologies. However, there are different views on what makes different technologies relevantly similar, and different ideas on what level of regulatory force should be equally applied on the similar set of technologies. The researchers note that biotechnology advocates apparently would like to see regulatory force to be uniformly relaxed, while biotechnology skeptics desire the opposite move.

This research is published in *Professionals in food chains* (Wageningen Academic Publishers) with the title "Technology neutrality and regulation of agricultural biotechnology".

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The precautionary principle should be handled with precaution

When we do not know whether something might be dangerous or not, it makes sense to be cautious. If a new technology promises some benefits but also raises concerns of unexpected but possibly disastrous consequences, the technology should be introduced stepwise with adequate safeguards – or perhaps not at all. This is the rationale behind the precautionary principle.

This is an established principle in policy and law, and there are many versions of it. One often mentioned version is the one found in the so-called Wingspread Statement:

When an activity raises threats of harm to human health or the environment, precautionary measures should be taken even if some cause and effect relationships are not fully established scientifically.

There have been plenty of critical objections to the precautionary principle over the years. Some claim that it is pointless to talk about 'the' precautionary principle, since there are several different versions, and, in addition, those versions are vague, that is, it is not clear what they mean and entail. This makes the principle difficult to apply, and to apply in a fair way. Another critique is that the precautionary principle is rigid and will lead to the undesirable situation where everything will be banned – after all, everything we do might turn out to have some severe unexpected consequences, however unlikely that outcome might be.

"Reasonable precautionary measures should be taken to safeguard against uncertain but non-negligible threats."

In a forthcoming article in the journal *Ethics, Policy & Environment* with the title "Is the precautionary principle a midlevel moral principle?", Per Sandin and Martin Peterson (Texas A&M University) deal with these problems and present a new way of conceiving the principle.

First, they argue that even if there is perhaps no 'core' that is common to all versions of the precautionary principle, it is plausible to think of the different versions as being related to each other by way of family resemblances – like a sister resembles her brother, who resembles their cousin, though the sister and the cousin do not resemble each other at all.

Second, they argue that the precautionary principle can be seen as a moral mid-level principle. Applications of such principles is today a commonly adopted approach in the ethics of medicine and health care. Mid-level principles do not offer any ultimate justification of moral judgments. Instead they are more flexible and must be balanced against other principles, as general guidelines from which more specific rules are formulated.

Peterson and Sandin's tentative version of a mid-level precautionary principle states: 'Reasonable precautionary measures should be taken to safeguard against uncertain but non-negligible threats'.

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In the EU the precautionary principle "may be invoked when a phenomenon, product or process may have a dangerous effect, identified by a scientific and objective evaluation, if this evaluation does not allow the risk to be determined with sufficient certainty.".

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Humans changing animal bodies – ethical issues beyond welfare

Animal welfare is not the sole ethical issue in livestock breeding, albeit important, but rather ethical agency and breeder integrity should be in focus. That is, responsibility for both an individual animals' welfare through creating a 'culture of care' and (given that the ethical sphere is wider than the welfare sphere) fostering an attitude of respect for, and the inherent value of, each individual. This conclusion is drawn by Helena Röcklinsberg in a study that was presented at the symposium "Changing animal bodies – animal breeding in changing social and environmental contexts" at Uppsala University, and will be published in the coming symposium book.

Breeding and selection for certain traits have been tools to change animals according to human needs and interests since the first dog was domesticated. This practice rests on the normative idea that we as humans have the right, and, some would argue, also the duty, to change nature, including animals, to our own benefit.

Modern advances in animal breeding technology and genetic modification opens for unforeseen possibilities and risks implying a need for ethical reflection. Further, it leads to a need to elaborate on more fundamental ethical issues such as what constitutes an 'animal' or what 'responsibility' or 'fairness' implies in relation to technologies, and, in the case of animals, whether there are wider ethical concerns than those related to welfare and whether all sentient beings or only a certain group of these are considered morally relevant, and then, according to what criteria do we make the distinction?

It is well known that selection for a certain trait can have a double effect. On the one hand it will, if successful, enhance the capacity or function selected for, but on the other it may simultaneously lead to compromised welfare of the animal, *e.g.* a large cow udder for increased production increases the risk of hurting the teats when raising from rest.

Above such evident welfare issues there might also be further ethically relevant issues to consider, such as whether the animal has an intrinsic value and/ or integrity, and if yes, if this leads to a set of rights corresponding with human obligations. Both these lines of thoughts, welfare and the intrinsic value cluster respectively, focus on the moral subject and criteria for establishing moral status, *i.e.* the animal and its qualities and capacities, and are important components in animal ethics. But, once we have clarified whom to care about, the question of moral agency remains.

Helena Röcklinsberg relates these fundamental issues to the set of issues connected to what motivates moral agency, or, what the core issue is when reflecting about breeding, on the basis of considering oneself morally responsible for animals involved. Not the moral subject, the animal, but ideas on what constitutes moral agency (*i.e.* relates to human responsibility) are in focus. Here the idea of shared vulnerability is relevant in relation to how we understand the situation of the animals, also in decisions on the use of newer breeding technologies such as genetic modification. Another aspect of moral agency in relation to our responsibility for animals is the view that we, as ethical agents 'in' an animal nature confer value to something, and thereby give it a reason to be strived for. In Christine Korsgaard's words, "To the extent that we value and disvalue these things, we are valuing our animal nature; and when we legislate for and against these things, we are legislating on behalf of our animal nature". These theories could facilitate a 'culture of care', long proposed in animal-based research, and highly relevant also in livestock breeding.

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Ever since (at least) Pythagoras 2500 years ago, there has been a continuous ethical reflection on the ideal human-animal relationship and the challenge from development of new practices, most recently through the advances in animal breeding technology and genetic modification.





Currently there is only one GM crop variety approved for commercial cultivation in the EU, the maize MON810. This variety is bred to be resistant against the European corn borer. It was approved in 1998.

Policy and legislation

All new crop varieties defined and regulated as genetically modified organisms (GMOs) in the EU have to go through a risk evaluation. After that the national representatives in the Standing Committee on Plants, Animals, Food and Feed (for food and feed use) or the Regulatory Committee 2001/18 (for cultivation) can approve the crop, if a qualified majority of them votes "yes". However, this qualified majority is rarely achieved, as many EU Member States do not follow the recommendations from the European Food Safety Authority (EFSA), *i.e.* the authority that makes the initial risk evaluation.

"Even in cases that have been scientifically assessed as safe, domestic interests and perceptions prevail, and therefore new GM crops are not approved for cultivation."

In spite of the rapid development within the field of biotechnology the legislation has basically been standing still. The system went even more into a deadlock in July 2018 when the European Court of Justice (CJEU) ruled that the mutagenesis techniques used for genome editing cannot be exempted from the GMO regulation.

According to critics, obstacles, obsolescence and contradictions characterize the EU legislative framework surrounding GMOs. During 2018 our researchers within Mistra Biotech have continued to study the policies and the legislation that affect the use of biotechnology in plant breeding.

UNDERSTANDING OF FOOD POLICY IN THE EU

The development of today's EU food policy started in the 1990s in response to a series of food crises and events such as the outbreak of the mad cow disease. These events unveiled various issues and provided an opportunity for a new discourse and a new approach to food regulation.

Indicatively, the interpretation of "science-based risk assessment" of food products, "uncertainty" and "transparency" has been contested and turned the debate about genetically modified (GM) crops to a food safety debate in the EU, which has been particularly intense. As a result, even in cases that have been scientifically assessed as safe, domestic interests and perceptions prevail, and therefore new GM crops are not approved for cultivation.

This evolution of the food policy in the EU has been examined by Sevasti Chatzopoulou. She concludes that the implementation of precaution and scientific





evidence has been dominated by intergovernmental policymaking characteristics. This demonstrates that the cognitive dimensions are significant in framing and reframing policy paradigms. The study emphasizes the complexity of the policy and unfolds the various related dimensions to food, directly or indirectly.

The study is to be published in *Encyclopedia of European Union Politics* with the title "The food policy of the European Union".

THE LACK OF CONSISTENCY

In the EU legal system, new varieties of crops are regulated differently depending on whether they are GM or not. The procedures and requirements for GM crops are much more stringent than those for non-GM crops. As confirmed by recent research, the GM/non-GM dichotomy is not an accurate indicator of health or environmental risks. Therefore, the regulatory divide cannot be justified from the viewpoint of risk assessment.

This is concluded by Karin Edvardsson Björnberg and colleagues in a study entitled "Consistent risk regulation? Differences in the European regulation of food crops", published in the *Journal of Risk Research*.

The special legal treatment of GM varieties originated in the 1970s when the researchers who first managed to produce recombinant DNA wanted to investigate the potential risks before proceeding with their research. Precaution was justified then, given the state of knowledge at the time. However, today our knowledge in genetics, plant biology and ecology has increased dramatically.

In the study the researchers use 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 based on ongoing plant breeding projects in Mistra Biotech. The researchers tested the relevance of the current legislation regarding risks by answering a number of questions about, for example, invasiveness and toxicity for the four cases. They conclude that invasiveness depends on the crop's ability to survive outside of the 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 glycoalkaloids. 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.

The researchers also conclude that the current GM crop legislation in the EU differs from several other risk-related legislations, *e.g.* regarding pesticides and

pharmaceuticals. The GMO legislation is based on an assessment that only considers risks, while in other legislations, risks are usually weighed against benefits. If risk/benefit trade-offs were considered in the GMO legislation, one would expect stricter requirements to be put on a (GM or conventional) crop that lacks substantial benefits for the public than on a GM crop

"From a biosafety point of view, is it relevant to regulate two indistinguishable crops in different ways, just because they are developed using different plant breeding methods?"

that has a substantial environmental benefit. Consumers may be against 'tampering with nature', or have other existential or religious grounds for rejecting GM products. Such concerns may justify a special regulatory treatment of GM varieties, but the legislation would then have to be tailored to deal with those particular issues. With a separation of issues, it would be possible to reconsider what type of legislation on agricultural crops is needed in order to prevent risks to health and the environment.

RANDOM OR TARGETED MUTATIONS – DOES IT MATTER?

Researchers and legislators should ask themselves "From a biosafety point of view, is it relevant to regulate two indistinguishable crops in different ways, just because they are developed using different plant breeding methods?" The question is raised by Dennis Eriksson who studies the EU regulatory framework for GMOs and its potential application also to crop varieties developed with newer plant breeding methods, such as genome editing through targeted mutations.

Currently crop varieties developed using old random mutation techniques are exempted from the GMO regulation. However, in July 2018 CJEU ruled that the strict GMO legislation should apply to plants developed with the new techniques for targeted mutagenesis, such as CRISPR/Cas9. There is no risk associated with the latter technique that justifies this difference and it is not possible to see any differences in the crop or the end-product if a genetic change has occurred randomly in the breeding process or if more targeted methods are the reason for the change (in the cases where no inserted DNA prevails in the genome).

The study is published in the journal *Theoretical* and *Applied Genetics* with the title "The evolving EU regulatory framework for precision breeding".

THE EU APPROACH COMPARED TO OTHER JURISDICTIONS

While the regulatory regime for GMOs in the EU also applies to new mutagenesis techniques, according to the judgement from the CJEU, the progress has taken other directions in several other parts of the world according to an analysis by Dennis Eriksson and colleagues.

While the EU focuses on the techniques as such, a case-by case approach is implemented by a number of other jurisdictions. In March 2018, the United States Department of Agriculture announced that it will not regulate genome edited plants as GMOs if the plant traits could have been developed through conventional breeding. And Canada has an altogether different approach with their *Plants with Novel Traits* regulation, with a focus on the novelty of the end product. The "novel traits" can be developed through different techniques, and GM plants are not automatically in scope.

In other words, the CJEU ruling adheres to a process-based approach while many other countries have an emphasis on the regulation of the resulting product (the new crop variety). This deviation results in a risk of asynchronous approvals around the world, and disruptions in international trade, unless an identity preservation system for products of directed mutagenesis can be established. The approaches to directed mutagenesis in the jurisdictions in the EU, Argentina, Australia, Brazil, Canada, Chile, Colombia and USA were included in the analysis.

The overview is published in the journal *New Phytologist* with the title "A comparison of the EU regulatory approach to directed mutagenesis with that of other jurisdictions, consequences for international trade and potential steps forward".

THE LEGISLATION SHOULD GET BACK ON THE SUSTAINABILITY TRACK

Legislators presented the early drafts for the legislative texts on GMOs three decades ago. The purpose was to be cautious about new technologies and to ensure that the environment and human health are not exposed to risks. In these documents from the late 1980s it is obvious that the original idea was also to acknowledge the traits and benefits of the new varieties of crops. A lot has happened since then, and according to an analysis by Dennis Eriksson, the original intentions of the EU legislation on GMOs should be revisited and to some extent recovered.

"Instead of protecting us, the GMO regulation has become an obstacle to applications that promote health and prevent environmental risks."

Genetically modified crops that have so far become food and animal feed, have proven to be just as safe to eat as crops bred by conventional methods such as cross breeding. Instead of protecting us, the GMO regulation has become an obstacle to applications that promote health and prevent environmental risks. According to Dennis Eriksson, policy makers need to consider the following more thoroughly:

- Focus on the organisms and products, instead of the technology.
- Update and adapt the directives regularly, in line with the technological progress in the field of biology.
- Acknowledge that the hitherto cultivated GM crops and products, during many years of cultivation and use, have not shown any health risks.
- Take into account all the potential benefits of different GMOs and products. That is, benefits for agriculture, the environment and the economy.

The analysis of the early legislative drafts was published in an opinion article in the journal *Frontiers in Bioengineering and Biotechnology* with the title "Recovering the original intentions of risk assessment and management of genetically modified organisms in the European Union".

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Information about GM products can actually change consumer's attitudes

Genetic modification (GM), have evoked strong (negative) attitudes among the general public, especially when related to food. Given the recent development in genome editing and other applications of biotechnology, it has become even more interesting to try to understand how different characteristics of information about the technologies (style, content and complexity) relate to the consumers attitudes.

In science communication about GMOs you often see persuasive messages, but they seldom result in an attitude change among those who are very opposed. Patrycja Sleboda and Carl Johan Lagerkvist tested if messages with different levels of information, which were matched with a person's attitude towards GMOs, led to attitude change. They found that strong informative messages changed attitudes in a positive direction, both among those who had a positive attitude from the start and among those who were strongly opposed to GM foods. The information frames, however, were personalized in such way that the strongly opposing group received a "lower risk" frame while the more positive group received a "higher benefits" frame. In line with previous research, they did not find any changes when the messages were non-informative, i.e. had weak arguments.

For example, "The new potato is perfect for those who watch their calories. You can eat it and be slim! The new potato is just as good as the conventional one and in addition it is very beneficial for keeping your body in a good shape" would not change the attitudes among the participants in the test, but the following message would do so: "A great benefit of the new potato, as scientist confirmed, is slower carbohydrates. That simply means that the energy we consume with the new potato stays longer in our body, and therefore, after eating it we feel full for a longer time not seeking for snacks. The composition of the new potato prolong satiation which itself is great for weight loss".

In more detail, the researchers first tested the participants' attitudes, their risk and benefit perceptions of GM-food in general, and their levels of acceptance of (willingness to buy) a potato genetically modified to be healthier, resistant to pests, or in less need of fertilization. Then the participants were given the messages about GMOs. Those who perceived GM food as high risk and had negative attitudes towards GM food were given a "lower risk" messages frame. On the other hand, those who were positive towards GM food and perceived it as low risk got a "higher benefits" message frame about the GM potato.

There are two "routes" or ways people process information which corresponds to the type of messages the participants received. The *central route* which involves a high level of cognition and careful analysis of the information presented. This relates to the strong informative message. The attitude changes based on the central route is assumed to be abiding and resistant, to last longer, and they seem to have more influence on our behavior. The *peripheral route*, which relies on general impressions related to positive and negative cues based on heuristics, is unrelated to logical reasoning. It relates to the weak informative message. The results will be published during 2019.

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Sweeping arguments about potential benefits from a specific GM crop do not seem to affect people's attitudes towards GMOs, but more informative messages might.





"We know that once shaped, attitudes are difficult to change. Especially if they are strongly negative."

– Patrycja Sleboda

She wants to know what changes your mind

It was not without doubt that Patrycja Sleboda decided to study psychology, and even during her first year of studies she was not sure if this subject was for her.

- All I heard from other students was how much they always dreamed about studying psychology, and I did not. The first year was very general and I questioned whether it would be something for me. I needed to know more. So, during the second year I took all the courses for both the second and third year. I had to study very hard, and I had so many exams, but at the end of that year I found myself very interested in human behavior and especially in decision-making psychology.

Patrycja got involved in research on risk perception and decision making through a professor who was her lecturer during the second year of studies.

- I loved her energy and could feel her passion and that definitely inspired me. Later on she became my master thesis adviser and then my PhD supervisor. The more knowledge I got, the more I loved it. Now, I cannot imagine doing anything else.

During her graduate studies, Patrycja focused on two main projects. One was devoted to public acceptance of known and unknown technologies with focus on emotional aspects of risk perception. The other was a more theoretical study on rational decision making and individual differences in information processing. Patrycja is now a PhD in psychology of decision-making.

Since the end of 2017 she has been working as a postdoctoral researcher at the Department of Economics at SLU, and is part of the Mistra Biotech programme.

– In Mistra Biotech I work on more applied research. My focus is on combining psychology and behavioral science to address behavioral and societal problems that concerns changing human behavior. Attitudes are often the required starting point to study sustained behavioral change. I focus on what kind of communication that affects peoples' attitudes towards GM food. It is very exciting to apply my theoretical knowledge, within attitude and behavior change through communication, on a real world situation, she says.

Patrycja grew up in the south of Poland and in Brooklyn, New York.

– I come from Sandomierz, an adorable town with an interesting history. My father moved to New York when I was seven and the rest of the family used to visit a lot before we all moved there some years later. I both loved and hated NYC. The city is always busy and offers a lot of opportunities. But it can be a bit overwhelming for a high school student. It is very competitive and you need to do many volunteer activities to get a chance at college, plus you are working as well.

So Patrycja decided to leave, against her parents' wishes. Now she feels that she belongs both to Europe and to the USA.

- I grew up in a European culture, I have a European mindset and definitely consider myself as Polish overall. At first I was very happy to come back, but then I started to miss NYC. I go there very often. Apart from my family my fiancé lives in NYC too. I think NYC is a very special place, once you get to love NYC it will always be a part of you.

As a postdoc in Mistra Biotech Patrycja has designed and conducted a successful study on effective communication and what changes peoples' attitudes towards genetically modified food (see page 28).

- It is very surprising people still believe that genetically modified food is risky and harmful. We know that once shaped, attitudes are difficult to change. Especially if they are strongly negative, she says.

A main problem, as studies show, is that people who are highly opposed to GMOs have low levels of knowledge about them.

- However, just presenting facts about products has not been found to be successful, says Patrycja.

She and her colleagues study how applied personalized communication, that matches risk and benefit perception of the participants in their experiment, affect the participants' attitudes.

All messages had to be presented in such way that the general public could understand them, but they were all designed based on scientific facts and with help and consultation of natural scientists.

- This multidisciplinary approach to research is the most exciting thing about working within Mistra Biotech. For me it is a great opportunity to learn completely new things from colleagues within the areas of plant breeding, ecology and economics.

Patrycja Sleboda

Dept. of Economics, SLU





"With time I have realized that science more resembles a puzzle that is slowly being laid out and that sometimes you might have to remove a piece."

– Henri<mark>k</mark> Svennerstam

He is giving the potato access to all the soil nitrogen

Forest and plants always attracted Henrik Svennerstam. His grandfather used to bring him along on different forest activities, picking berries, fishing, and even cutting down some trees. Years later a school project run by the Faculty of Forest Sciences at SLU in Umeå gave him an insight into the forest research at the university – the first step towards becoming a scientist. Although at the time he had a different view of the research process.

- To begin with I thought of science as something rather digital, either something is, or isn't. But with time I have realized that science more resembles a puzzle that is slowly being laid out and that sometimes you might have to remove a piece, since it turns out to be in the wrong place, he says.

Henrik grew up in a village not far from Sundsvall and moved to Umeå to study at SLU. He has a Master of science in forestry and a PhD in plant physiology and is one of Mistra Biotech's researchers who develop new potato varieties with increased nitrogen use efficiency.

- In laboratory experiments microorganisms such as bacteria and fungi are often found to be better competitors for nutrients than plants. If that was also true in nature, with microorganisms winning the race for resources, our ecosystems would be dominated by them. But if we look around, we see a planet covered with plants. For me this is an example of the complexity and challenging nature of research, especially in the context of experimental design and the interpretation of results, he says.

The research group Henrik belongs to has a long history of plant research related to nitrogen. One of their topics is the importance of soil organic nitrogen for plants. During his PhD studies, they identified two amino acid transporters crucial for *Arabidopsis thaliana* root amino acid uptake. These transporters account for the absolute majority of root amino acid uptake in the plant, and the group was able to demonstrate a correlation between amino acid uptake capacity and plant growth.

- The breakthrough moment was when I found a single surviving plant in a screening experiment using an *Arabidopsis* knockout library. The line was carrying a mutation in a specific amino acid transporter and the following work characterizing it resulted in the major part of my doctoral thesis. We were the first group to succeed in improving plant nutrient uptake, says Henrik. Within Mistra Biotech his aim is to transfer the knowledge from the model species *Arabidopsis* into potato. By using *Arabidopsis* lines with low and high amino acid uptake capacity he can investigate the effect of the uptake capacity on the microbes in the rhizosphere (the soil surrounding the roots).

- The rhizosphere is where roots and soil microorganisms co-exist and they may have a direct influence on each other. We want to investigate if decreased or increased plant amino acid uptake capacity will change the soil nutrient dynamics and as a consequence alter the microbial composition in the rhizosphere, says Henrik.

The production of industrial fertilizers such as ammonium nitrate has had a monumental impact on agriculture globally. It is estimated that 50 percent of the world population is supported by synthetic fertilizers. However, since plants do not always have the capacity to fully utilize the applied fertilizers nitrogen leaching may occur, so the use of synthetic fertilizers is thus not without problems.

Organic nitrogen on the other hand is the product of decomposing organic matter.

- By increasing the amino acid uptake capacity, the potato should become more efficient in using the available organic nitrogen pool and, as a consequence, possibly also have less need for synthetic fertilizers. But even if we succeed, the potato still has to be fertilized, but the form(s) of nitrogen and management may be different.

Henrik has been a part of Mistra Biotech from the start of the programme in 2012.

- Working with potato within this programme is a great opportunity to transfer the basic research carried out in *Arabidopsis* to an agricultural crop of economic and societal value, which in the end I believe is the ultimate goal of science. In the context of biotech and genetic modification, the interdisciplinary nature of Mistra Biotech has given me invaluable insights into policy making and regulations and raised my awareness about consumer attitudes and ethics.

Henrik Svennerstam

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Dept. of Forest Genetics and Plant Physiology, SLU

Shaping our food – a podcast on crop and livestock breeding

How can a nasty fungus be prevented from attacking a plant? Is it possible to breed sows to not lie on their piglets? How do researchers change the genes of plants and animals? And what are the criteria for ethically right and wrong? These are some of the questions discussed when researchers and experts, from both within and outside Mistra Biotech are invited to explain the science surrounding breeding and biotech. The podcast has so far published eleven episodes (in Swedish).

- # 1. Varför behöver vi växtförädling?
- # 2. Varför behöver vi djuravel?
- # 3. Att tämja en vild växt
- # 4. Kampen mot algsvampen
- # 5. Havrens bästa tid är nu
- # 6. Hundra procent onaturligt
- # 7. Guld och gröna åkrar, inte gröna hav
- # 8. Proteinerna tar oss närmare sanningen
- # 9. Noas ark på riktigt genbanker bevarar egenskaper för framtiden
- # 10. Ett krämigt, såsigt, fast ändå stabilt avsnitt om stärkelse
- # 11. En hel massa om arvsmassan genomikvägen till egenskaperna

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E-MAIL US

In addition to spreading the knowledge we have gained within Mistra Biotech, we want to have a dialogue with you. It is valuable for us to learn more about people's opinions and views on biotechnology in agriculture. It is also important for us to take part of the knowledge among stakeholders and other researchers. Do you have a question or something you wish to share with us? Do you want to get in contact with one of our researchers in a specific area?

Please send us an e-mail to mistrabiotech@slu.se





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Aakash Chawade Carl Johan Lagerkvist Catja Selga Cecilia Gustafsson Charlotta Zetterberg Dennis Eriksson Dirk-Jan de Koning Elena Flavia Mouresan Elisabeth Jonas Emelie Ivarson Erik Alexandersson Erik Andreasson Fernando Lopes Pinto Fredrik Levander Fredrik Reslow Freddy Fikse Helena Röcklinsberg Henrik Svennerstam Inger Åhman

Department/Company

Plant Breeding, SLU Economics, SLU Plant Breeding, SLU Plant Breeding, SLU Department of Law, Uppsala University Plant Breeding, SLU Animal Breeding and Genetics, SLU Animal Breeding and Genetics, SLU Animal Breeding and Genetics, SLU Plant Breeding, SLU Plant Protection Biology, SLU Plant Protection Biology, SLU Animal Breeding and Genetics, SLU Immunotechnology, Lund University Plant Breeding, SLU Växa Sverige Animal Environment and Health, SLU Forest Genetics and Plant Physiology, SLU Plant Breeding, SLU



Name

Jakob Willforss Jane Morrell Julie Clasen Karin Edvardsson Björnberg Klara Fischer Lars Rönnegård Lena Dimberg Li-Hua Zhu Maria Selle Mariette Andersson Marit Lenman Mattias Eriksson Mattias Holmlund Micaela Maria Kulesz Mickey Gjerris Muhammad Awais Zahid Mulatu Geleta Dida Patrice Humblot Per Sandin Pernilla Vallenback Patrycja Sleboda Ranjan Ghosh Rodomiro Ortiz Roger Andersson Sebastian Hess Selvaraju Kanagarajan Sevasti Chatzopoulou Shyam Kumar Basnet Sjur Sandgrind Sreten Andonov Sung-Yong Kim Svante Resjö Sven Ove Hansson Torgny Näsholm Ulrika Ganeteg Xue Zhao Zeratsion Abera Desta

Department/Company

Immunotechnology, Lund University Clinical Sciences, SLU Animal Breeding and Genetics, SLU Philosophy and History of Technology, KTH* Urban and rural development, SLU Animal Breeding and Genetics, SLU Molecular Sciences, SLU Plant Breeding, SLU Mathematical Sciences, Norwegian University of Science and Technology Plant Breeding, SLU Plant Protection Biology, SLU Energy and Technology, SLU Forest Genetics and Plant Physiology, SLU Economics, SLU Food and Resource, University of Copenhagen Plant Protection Biology, SLU Plant Breeding, SLU Clinical Sciences, SLU Crop Production Ecology, SLU Lantmännen Lantbruk Economics, SLU Centre for Management in Agriculture, Indian Institute of Management Ahmedabad Plant Breeding, SLU Molecular Sciences, SLU Dairy and Food Industry Economics, Kiel University Plant Breeding, SLU Society and Globalisation, Roskilde University Economics, SLU Plant Breeding, SLU Animal Breeding and Genetics, SLU Plant Breeding, SLU Plant Protection Biology, SLU Philosophy and History of Technology, KTH* Forest Ecology and Management, SLU Forest Genetics and Plant Physiology, SLU Molecular Science, SLU Plant Breeding, SLU

* Royal Institute of Technology (KTH)



















Activities

28/1-1/2 Mariette Andersson gave a talk on "CRISPR-Cas9 takes several bites in the potato genome- efficient targeted multiallelic mutagenesis in tetraploid *Solanum tuberosum*" at the Keystone Symposia on molecular and cellular biology, USA.

7-8/2 M. Andersson presented "En revolution inom växtförädlingen" at the Nordic Agron Summit, Malmö.

12/2 Poster presentation "Genomic prediction including SNP-specific variance predictors" by Elena Flavia Mouresan et al. at the World Congress on Genetics Applied to Livestock Production in Auckland, New Zealand.

8-10/3 Mistra Biotech participated at SciFest 2018 with live podcasts and in the SLU Future Food stand.



Per Sandin talking to school children at the SciFest at Fyrishov in Uppsala.

12-14/3 Dennis Eriksson was invited to give a talk on "The global regulatory landscape for plant genome editing – hurdles and opportunities for breeding to reduce mycotoxins" at the World Mycotoxin Forum in Amsterdam, NL. 22-23/3 Mistra Biotech Annual programme meeting.



Programme Director Sven Ove Hansson listening to a group discussion at the annual Mistra Biotech meeting.

22-24/2 Erik Andreasson gave a talk "Biotic stress resilience in potato" at the Plant and molecular biology conference, Paris, France.

26-27/3 D. Eriksson was invited to give a talk at the GARNet-BCAI workshop on genome editing, Bristol, UK.

26/4 Karin Edvardsson Björnberg and Charlotta Zetterberg talked about "GMO and product- or process-based legislation" at the Swedish Environmental Protection Agency.

2/5 K. E. Björnberg and C. Zetterberg gave a presentation on "GMO and product- or process-based legislation" at the Swedish Gene Technology Advisory Board.

2/5 Mickey Gjerris was part of the panel at the ethical discussion on "Findes der 'naturlige' fødevarer? Og er alle typer af genmodifikation uforenelige med forbrugernes ønske om sunde og bæredygtige fødevarer?" at the debate "Hvordan får vi et bæredygtigt landbrug i fremtiden – kan det lykkes uden GMO?" arranged by The Danish Council on Ethics and The Norwegian Biotechnology Advisory Board in Copenhagen.

8/5 D. Eriksson presented "Hur ser EU och dess medlemsstater på CRISPR/Cas?" the seminar "Regelverket kring växtförädlingen", at the The Royal Swedish Academy of Agriculture and Forestry, Stockholm. **8/5** Helena Röcklinsberg gave a presentation "Humans changing animal bodies: issues beyond welfare" at the Changing Animal Bodies Symposium, Uppsala University.

14-15/5 Anna Lehrman & Klara Fischer attended "Where is life science heading in the future? Genes, technology and society" organized by Stockholm Science City Foundation and Axel and Margaret Ax:son Johnson Foundation at Engelsbergs bruk, Fagersta.

15-18/5 H. Röcklinsberg gave a talk "Insects for food and feed – four challenges" at the conference "Insects to feed the world", Wuhan, China.

27-30/5 Poster "Relationships between the content of seminal plasma proteins and bull fertility" by Jane Morrell et al. at the conference "Bull fertility- theory to practice", British Society for Animal Science, Westport, Ireland.

1/6 Per Sandin was part of the panel at the AGFO talk discussing "Hur förändras maten?".



Both Per Sandin and Carl Johan Lagerkvist have been invited as panelists at different AGFO talks.

13-16/6 P. Sandin, H. Röcklinsberg, K. E. Björnberg and M. Gjerris organized a workshop "Regulating Plant Biotech 2.0: CRISPR potato as a case study", during the EurSafe Congress in Vienna, Austria.
D. Eriksson was invited as one of the discussants.
K. E. Björnberg gave the presentation "Putting the CRISPR potato on the table – legal and ethical challenges" and was also part of a panel discussion.
P. Sandin acted as moderator and panel chair during the workshop. H. Röcklinsberg gave a presentation

"Potato crisps from CRISPR-Cas9 modification – aspects of autonomy and fairness", M. Gjerris talked about "Could crispy crickets be CRISPR-Cas9 crickets – ethical aspects of using new breeding technologies in intensive insect-production", and P. Sandin gave a presentation on "Technology neutrality and regulation of agricultural biotechnology". P. Sandin, K. E. Björnberg (and Christian Munthe) gave a presentation under the title "Understanding technology neutrality in regulation of agricultural biotechnology" and H. Röcklinsberg and M. Gjerris presented "Potato Crisps from CRISPR-Cas9 modification – freedom to choose or free from choice?" at the same congress.

14-15/6 M. Andersson and Board member Stefan Jansson had a stand showcasing CRISPR-edited crops at the "FOOD 2030 High level event: Research and Innovation for Food and Nutrition Security – Transforming our food systems", Plovdiv, Bulgaria.



Mariette Andersson challenging participants at the Food 2030 in Plovdiv to guess which plants had been genome edited.

18-21/6 Cecilia Gustafsson presented a poster "Accelerating the domestication of a novel oilseed crop through genomics application" at the Plant Biology Europe conference in Copenhagen.





27-28/6 Mistra Biotech in general and the podcast Shaping our food in particular was part of the SLU stand at Borgeby Fältdagar outside Lund.



Lisa Beste interviewing a visitor at the Borgeby Fältdagar agricultural fair.

3/7 A. Lehrman was invited panelist at the seminar "Fake eller fakta om mat" arranged by SLU Future Food at the Almedalen week in Visby.



Anna Lehrman was one of the panelists at the discussion about "fake or facts" about food arranged by SLU Future Food in Almedalen.

22/7 Patrycja Sleboda gave a talk "Persuasive messages and attitude change towards genetically modified food" at the SABE/IAREP conference, International Association for Research in Economic Psychology, London, UK.

25/8 Mistra Biotech had a stand at the SLU food fair Matologi in Stockholm, showcasing field cress and potato plants and products and challenging. Catja Selga and C. Gustafsson talked about the research and challenged visitors in a plant breeding quiz. A. Lehrman was interviewed for the podcast Matlaboratoriet and P. Sandin was part of the discussion panel on future food.



Catja Selga in the front, and Cecilia Gustafsson in the back, talking to visitors at the Matologi event in Stockholm.

30/8 Carl Johan Lagerkvist was invited as panelist at AGFO talk, "Hur väljer vi maten?".

3-5/9 M. Andersson gave a talk "Transgene-free CRISPR-Cas9 inducing multiallelic mutations in tetraploid potato (*Solanum tuberosum*)" at the cenference "CRISPRing – A new beginning for the genetic improvement of plants and microbes", Budapest, Hungary.

7/9 A. Lehrman & P. Sandin was invited to a workshop at Spritmuseum in Stockholm which was a part of the planning for the "Framtidens mat & dryck" exhibition running from February until October 2019.

3-5/9 C. Selga et al. presented a poster "Low-cost genotyping for potato breeding" at the 17th meeting of the EUCARPIA Section Biometrics in Plant Breeding, Ghent, Belgium.

10-12/9 E. Andreasson gave a talk "Comparative proteomics of three different immunity reactions in potato" at the INPPO Conference, Padova, Italy.

19/9 K. Fischer and S. Jansson held an open lecture and seminar at Örebro University on the topic "Global livsmedelsförsörjning – vilken roll kan GM-grödor spela?".

18-21/9 Sevasti Chatzopoulou participated as a stakeholder at the EFSA annual conference "Science, Food, Society", Parma, Italy.

22/9 P. Sandin was part of the panel on the session "Hacking Life" at the symposium "Limits of life" organised by iGEM, Ångström lab, Uppsala.



2/10 The HVO (Hydrogenated vegetable oils) manufacturer SunPine visited Alnarp and C. Gustafsson gave a presentation on field cress.

15/10 M. Andersson was invited to "Innovation in agriculture: Women pioneers at the frontiers of science" held at the European Parliament, organized by United States Mission to the European Union together with the European Parliament, S&D and EPP. She gave the talk "A hot potato-CRISPR crops for improved nutrition and environmental performance", Brussels, Belgium.

16/10 K. Fischer was invited by the Norwegian Biotechnology Advisory Board to give a talk, "Socioeconomic aspects of GMO in Africa", at the open meeting on GMO in Africa. Oslo, Norway.



Klara Fisher was invited as a speaker at a meeting about GMO in Africa arranged by the Norwegian Biotechnology Advisory Board.

17/11 K. E. Björnberg gave a presentation, "EU:s GMO-lagstiftning - en regulatorisk återvändsgränd?" at Bioteknikdagarna, KTH.

18/12 P. Sleboda presented a poster "Persuasive messages and attitude change towards genetically modified food" at the Society for judgment and decision making's 39th Annual conference, New Orleans, USA.

2-3/12 Jakob Willforss presented a poster entitled "Countering technical bias and batch effects in proteomics" at the 17th Swedish Proteomics Society meeting in Lund.

4-6/12 C. Selga gave a presentation entitled "Exploring genomic-estimated breeding values for selection in a small sized potato breeding program for Nordic Europe" at the 19th Joint meeting of the Section breeding & varietal assessment of the European association for potato research and the EUCARPIA section potatoes. Gatersleben, Germany.



INTERNATIONAL COLLABORATIONS ON PLANT GENOME EDITING

Mistra Biotech researcher 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. The project *PlantEd – Plant genome editing, a technology with transformative potential* will guide and facilitate applications of genome editing in plant research and breeding, which in turn will help setting R&D priorities and stimulating further cross-national and cross-disciplinary collaborations.

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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



Mistra Biotech in the media

2/1 Gene study: Scientists need to tell their stories, The Country Today

5/1 Genetic modification and genome editing rely on active roles for researchers and industry, Cowsmopolitan

15/1 Så vill forskare öppna för mer GMO i EU, Aktuell Hållbarhet

22/1 Genmodifierad soja konkurrent till svenska proteingrödor, Land Lantbruk



A publication from 2017 that gained media attention was the study on potential effects if Sweden was to import GM soy.

25/1 Ny hälsosammare potatis upptäckt, Folkbladet

25/1 SLU: Ny potatis för alla som gillar långsamma kolhydrater, Food Monitor

25/1 Ny hälsosammare potatis upptäckt, Västerbottens-Kuriren

28/1 Sju av tio tror att GMO finns i butikerna, TT, DI, Sydsvenskan, Helsingborgs Dagblad et al.

29/1 Ny sorts potatis framtagen på SLU, P4 Uppland and Göteborg, Sveriges Radio

29/1 Genförändrad potatis får extra fibrer, Forskning & Framsteg

29/1 SLU: Här är den nya "hälsopotatisen" – som ska få fart på magen, Metro and MSN Livsstil

29/1 Många svenskar tror att butikerna har GMO-livsmedel, Food Supply, and Fri Köpenskap





Xue Zhao's study on dietary fiber in high amylose potato generated broad media attention.

30/1 Ny potatis med långsamma kolhydrater, Statskoll.se

30/1 Ny potatis kan bli hälsovinnare, ATL

31/1 Många saknar kunskap om GMO-livsmedel, Land Lantbruk

31/1 Hälsoboosta potatisen: 3 enkla metoder, Land

6 & 7/2 Svenska forskare har skapat en mer hälsosam potatis, Allas & Året Runt

7/2 Svenska forskare har gjort potatis nyttigare, MåBra

7/2 "Europas länder bör själva få bestämma om GMO", Mistra

11/2 SLU: GM-fri soja förstärker klimathotet, ATL

11/2 GM-soja eller GMO-fri soja? Jordbruksaktuellt

16/2 Så ska våra grödor klara sig med stigande temperatur, SVT Vetenskap

17/2 De genmodifierar växter – för att klara global uppvärmning, Expressen

20/2 GM-soja eller GM-fri soja – påverkan på miljön, Grisföretagaren





Dirk-Jan de Koning commented on Bill Gates' expectations on the genome editing tool CRISPR/Cas 9 and its capacity to transform livestock breeding, in the radio programme Vetenskapsradion.

12/3 Potatisens stärkelse, Lantbruksnytt

23/3 Wheat in heat: the 'crazy idea' that could combat food insecurity, The Guardian

26/4 De skapar växter som är resistenta mot bladlöss, SVT

2/5 Nytt från Mistra Biotech: Avelsbranschen behöver etiska kommittéer för genteknik, Mistra

22/6 Forskare har hittat 30 arvsanlag som ska ge ny oljeväxt bra egenskaper, Industripress

2/7 Bill Gates: Crispr/cas9 kan förändra den globala utvecklingen, Vetenskapsradion

5/7 Framsteg i förädlingen av ny oljeväxt, Jordbruksaktuellt



The progress in the domestication of field cress is gaining more and more interest in the media.

8/8 Klimatförändringar tvingar fram nya grödor, SVT Skåne

Sept. Sommarens EU-dom slår hårt mot Öresundsregionens forskning, Rapidus

3/9 GMO-beslut drabbar forskningen, Lunds Universitet

16/9 Bonden och handeln kan påverka konsumenternas val, Land Lantbruk

Nr 10 Framtidens drivmedel görs på fältkrassing, Lantmannen

20/9 Forskare: EU-beslutet hotar växtforskning, Ny Teknik



The ruling by the European Court of Justice that genome edited plants should be regulated as GMOs has been, and still is, debated.

8/10 Öka GMO-valfriheten inom EU!, Europakommentaren Lunds Universitet



Publications

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Burra, D.D. et al. 2018. Comparative membrane-associated proteomics of three different immune reactions in potato. *Int. J. Mol. Sci.* 19:538

Edvardsson Björnberg, K. et al. 2018. Consistent risk regulation? Differences in the European regulation of food crops. *J. Risk Res.* doi: 10.1080/13669877.2018.1501594

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Eriksson, D. 2018. Recovering the original intentions of risk assessment and management of genetically modified organisms in the European Union. *Front. Bioeng. Biotechnol.* doi: 10.3389/fbioe.2018.00052

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Gamborg C. et al. 2018. Could crispy crickets be CRISPR-Cas9 crickets – ethical aspects of using new breeding technologies in intensive insect-production. In: Springer, S. & Grimm, H. (eds.) *Professionals in food chains*. Wageningen Academic Publishers, the Netherlands. pp. 424-429

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Hansson, S.O. et al. 2018. Breeding for public health: A strategy. *Trends Food Sci. Technol.* 80: 131-140

Jonas E. et al. 2018. Genomic selection. In: Rajora O. (ed.) Population Genomics. Springer, Cham. pp 427-480

Lankinen, Å. et al. 2018. Plant immunity in natural populations and agricultural fields: Low presence of pathogenesis-related proteins in *Solanum* leaves. *PLoS ONE* 13: e0207253

Mesbah-Uddin, M. et al. 2018. Genome-wide mapping of large deletions and their population-genetic properties in dairy cattle. *DNA Res.* 25: 49-59

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Sandin. P. 2018. Review of A. Kallhoff, M. Di Paola and M. Schörgenhumer (eds.) Plant ethics: Concepts and applications. *Environ. Ethics* 40: 95-96

Sandin, P., Munthe, C. & Edvardsson Björnberg, K. 2018. Technology neutrality and regulation of agricultural biotechnology. In Springer, S. & Grimm, H. (Eds.) *Professionals in food chains*. Wageningen Academic Publishers pp: 223-228, ISBN: 978-90-8686-321-1

Ulén, B. & Aronsson, H. 2018. Nitrogen and phosphorus leaching under the potential biennial oilseed plant *Lepidium campestre* L. in a field trial. *Acta Agric. Scand. B Soil Plant Sci.* 68: 555-561

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