FGU
Project Genetic
Engineering and
the Environment

Background: Part 1 - Risks

Possible CRISPR/Cas effects on the environment

Rapidly developing new genome editing techniques signify an increasingly urgent need to robustly assess associated risks. Currently, the most frequently used and most promising method is the CRISPR/Cas system. This background paper discusses the possible effects of genome-edited plants on the environment and includes a case study on *Camelina sativa*, an annual plant mostly cultivated in Europe and in North America. The fatty acid content of camelina has already been changed several times using CRISPR/Cas9. The case study provides an explanation of unintended effects on the metabolic pathways of the genome-edited plants and outlines possible unintended environmental effects.

Unexpected and unintended effects from intended changes

Even if the changes in the DNA mediated by the application of genome editing are successful, the effects of these changes on the organism as a whole can be completely different to what is intended. Precision must not be equated with safety in this respect. Interactions with other metabolic pathways can, for example, change the composition of the ingredients in plants or make them more susceptible to diseases. Furthermore, interactions with pollinators, soil organisms or the food chain can also be affected.

Some of these effects are difficult to detect because it is not sufficient to just examine the DNA sequence. Instead, complex metabolic processes in the cell often have to be examined more closely.

Unwanted effects on metabolic and signaling pathways other than those intended

Apart from the intended changes, CRISPR/Cas can intervene in other signaling or metabolic pathways. This is because metabolic pathways are interconnected. DNA, RNA, proteins and/or metabolic products can interact and thereby stimulate or block particular functions. In cases where, for example, the gene scissors are used to prevent a gene from being activated and the corresponding protein is no longer produced, this can, in addition to the intended effects, also lead to a disruption of other signaling pathways in the cell. As a result, there might be an increase in the formation of a metabolic product that should not have been changed. The induced changes should never be considered on their own, but rather in the context of a complex, balanced biological system.

Unwanted environmental effects of genome-edited plants

Plants interact with many other species in their respective ecosystems. These can either be plants from their own species or from a different species. In addition, plants are part of a food web and interact with various animals. Plants are colonized by specific microorganisms both above and below ground. This is known as the plant microbiome.
External climatic conditions, such as drought, fluctuating temperature conditions or pest infestation, influence how the plant develops and can trigger stress responses in the plant. These environmental factors can cause processes within the plant which activate or silence genes in response to the respective stress situation and for their protection.

The intended changes in the genome of genome-edited plants can also inadvertently affect other metabolic pathways. This can trigger unwanted effects in the genome-edited plants, some of which may only manifest in certain environmental stress conditions or in later generations. The intended changes in genome-edited plants can also lead to unwanted environmental effects if the side effects interfere with interactions between the plants and their ecosystem.

Case study: *Camelina sativa*

This background paper uses a specific example to explain possible impacts that can occur when genome-edited plants are released into the natural environment. *Camelina sativa*, which has already been modified several times with CRISPR/Cas, was chosen for this purpose. Camelina is hexaploid, i.e. it has a six-fold set of chromosomes. It has been altered using CRISPR/Cas SDN-1 applications in a way that was previously not possible using conventional methods [1-3]. Camelina is rich in polyunsaturated fatty acids such as linoleic and linolenic acids. The proportion of monounsaturated oleic acid in the seeds was altered with the gene scissors, thus reducing the proportion of easily oxidizable polyunsaturated fatty acids [1; 2]. This is supposed to extend the shelf life of the camelina oil extracted from the seeds. The gene scissors were used to simultaneously knock out up to 18 copies of three genes in the genome of the camelina in order to produce plants with a higher oleic acid content. Such interventions were near to impossible to achieve using conventional methods. The genome edited plants may, however, have new biological traits. The change in both the fatty acid metabolism and the composition of fatty acids in the camelina plants is used below as an example to illustrate the possible effects on the genome-edited plant as well as its environmental impact.

Changes in the composition of fatty acids can influence the stress response

Fatty acids perform various functions in the plant cell and are involved in various processes. Polyunsaturated fatty acids, such as linolenic acid, are, for example, components of membranes in the cell (e.g. the cell membrane or the membrane of chloroplasts) and regulate both cell transport processes and the fluidity of the membranes. The fluidity of cell membranes is particularly important in changing environmental conditions, especially cold. The colder it is, the higher the proportion of unsaturated fatty acids in the cell membrane should be to keep the membrane fluid. Findings in Arabidopsis, a close relative of camelina, show that plants lacking a specific gene in fatty acid metabolism are unable to survive cold stress [4].

In camelina, it was shown that genes involved in fatty acid metabolism were expressed differently in response to a higher salt content in the soil, and therefore formed different gene products [5]. As a result, the composition of the membrane lipids and the fluidity of membranes was changed; this shows that plants can adapt to stress conditions by changing their gene activity.

In addition, long-chain fatty acids are components and precursors for other fatty acids in the cuticle and other molecules, such as waxes, which serve as protective boundaries of plants (e.g. in the root or seed coat). In camelina, waxes mainly regulate drought tolerance [6]. Interfering with plant fatty acid metabolism may, therefore, have unintended effects on the genome-edited plant under abiotic stress conditions such as drought and heat.

Change in fatty acid composition can influence the formation of messenger substances

Fatty acids often serve as starting molecules for the formation of various messenger substances in plants. These messenger substances are used, for example, for communication between individuals of the same species or other species to warn each other of pests. Such messenger substances are, e.g. so-called phytohormones and volatile organic substances.

Phytohormones act as messenger substances in plants, facilitating a response to external conditions. One example is the phytohormone jasmonic acid (JA), which is also important for growth. Plants release JA into the environment when attacked by pests, where it acts as a phytohormone on other plants [7].

JA is formed from linolenic acid, which comes from the cell membrane of plants [8]. The conversion of linolenic acid into JA involves several different metabolic steps. JA, in turn, then serves as a starting molecule that can be biochemically modified. Such substances are referred to as derivatives of JA, which, among other things, also act as messenger substances.

A soybean study shows what can happen when fewer unsaturated fatty acids are converted to JA in plants and, accordingly, less JA is available [9]: soybeans infested by soybean aphids contain less linolenic acid, which means that less JA can be formed. JA normally serves as a messenger that attracts ladybugs, which are natural predators of the aphids. The aphids block the activity of a very specific gene that regulates the formation of linolenic acid via a yet unknown mechanism. As a result, less JA and volatile organic compounds are formed in the soybean, which serve as the plants' natural defense response to attract more aphid predators. This allows more aphids to infest the soybeans.

CRISPR/Cas is used in camelina to knock out genes and reduce the content of linoleic and linolenic acids in the genome-edited plants. It is likely that this may also impact the formation of various signaling substances in camelina, thus affecting communication between different plants. Such effects often only become apparent when plants are exposed to various stress situations, such as a pest infestation; therefore, they need to be taken into account in risk assessment. Under natural conditions, plants are also often exposed to multiple stress conditions simultaneously, e.g. a pest attack in drought conditions. Such scenarios should be studied in more detail, as plants may regulate different metabolic pathways differently in certain stress conditions, which might have been affected by the modification of the target genes induced by genome editing.

Interference with the food web

Various interconnected food chains exist in an ecosystem, which in their entirety form a complex food web. Different organisms (including plants, herbivores and carnivores) feed on each other and are all part of a food web. If one organism and its characteristics are changed in a food chain, this will also affect other organisms in the food web. For example, if a plant that is a food source for a particular insect is modified by CRISPR/Cas and it then becomes toxic to that insect, the insect will die after ingesting the plant. This insect would then no longer be available as a food source for other animals, most likely disrupting the food web.

Fats are involved in many processes in plants and animals, including the formation of important messenger substances, but also in the supply of energy. Animals and humans cannot produce polyunsaturated fatty acids themselves and have to ingest them with their food [10]. Honey bee research has shown the effects altered food sources can have. In a study, honey bees were fed a diet containing no linolenic acid or other omega-3 fatty acids [11]. The bees had less polyunsaturated fatty acids in their bodies, and their brains and hypopharyngeal glands were smaller than bees fed with normal diets. The hypopharyngeal gland is where the foraging sap in the workers' heads is produced, which is in turn used to feed other bees. The bees in the study were less able to learn in various tests compared to bees that ate a normal diet.

Various species of bees and flies are among the insects attracted to the nectar and pollen of camelina [12-14]. Thus, altered composition of fatty acids due to changes induced by the gene scissors in the fatty acid metabolism of camelina may also affect food webs in which camelina is involved. This would be an unintended consequence for the ecosystem with consequences for other animals that would be difficult to foresee.

Genome-edited camelina could persist in the environment and spread uncontrollably

If genome-edited camelina with a lower concentration of polyunsaturated fatty acids is released into the environment, it can cross with wild species and invade new habitats. For example, it may interbreed with species such as hairy false flax (*Camelina microcarpa*) or common shepherd's purse (*Capsella bursa-pastoris*). This may result in unintended effects in subsequent generations. If the genome-edited camelina hybridizes with wild species, the progeny may gain a selection advantage and thus gain an advantage over wild species, and subsequently displace them. The unintended effects in the progeny may be significantly different from those originally observed in the laboratory.

Which methods can be used to analyze the composition of genome-edited plants?

There are a number of methods that can be used to analyze the composition and changes in metabolites in cells. These methods are collectively described as metabolomics. Metabolites, such as sugars, fatty acids, amino acids, alcohols, etc., are diverse and occur in various amounts within an organism. Metabolomics techniques have to meet complex analytical challenges in order to investigate the composition and amounts of these metabolites. Mass spectrometry and nuclear magnetic resonance spectroscopy techniques are primarily used for this purpose.

Metabolomics techniques allow molecules involved in metabolic processes to be detected within a cell and enable comparison with a corresponding reference. If a change leads to unforeseen effects in metabolism, an imbalance can occur and the formation of metabolic products can be disturbed in plants. This can have an impact on the genome-edited organism itself, but also on other organisms in the ecosystem. A metabolomics analysis is extensive and can break down the composition of precursor molecules, intermediates, end products and their derivatives in many different metabolic pathways. The more information is known about the altered metabolic pathway, the easier and more targeted the metabolite composition can be analyzed and, if necessary, the detection of specific metabolites. If, for example, the gene scissors interfere with fatty acid metabolism and an enzyme in the formation of a specific fatty acid is switched off, then the composition of all cellular lipids can be specifically investigated using lipidomics techniques (i.e. a spectrum of analytic methods within metabolomics). If it is not known which target gene and thus which metabolic pathway was altered, then an untargeted metabolomics analysis must be performed that is not designed to target a specific metabolite species (e.g., lipids, sugars, amino acids, etc.). The analysis is then broader in its scope, but less comprehensive in the different metabolite species. Metabolomics has been proposed several times for the evaluation of genome-edited plants [15; 16] and should be used in risk assessment.

It is particularly important to note that genome-edited plants with many different properties could be released if certain gene scissor applications are deregulated. In many cases, these genome-edited plants contain completely new traits that could not be achieved using conventional methods. Currently, it is very difficult to assess what environmental impact such genome-edited plants might have. Therefore, novel environmental effects of genome-edited plants must be investigated in risk assessment.

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