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Differences between conventional breeding and genetic engineering: An assessment of the statement made by the Group of Chief Scientific Advisors' (SAM)

Testbiotech e. V.
Institute for Independent Impact Assessment in Biotechnology

Christoph Then & Astrid Österreicher

Introduction

Testbiotech became aware of the 'Statement by the Group of Chief Scientific Advisors' (SAM), titled: **"A Scientific Perspective on the Regulatory Status of Products Derived from Gene Editing and the Implications for the GMO Directive"** (SAM 2018). After detailed analysis, we concluded that this statement needs to be revised. Many of the arguments used in the SAM (2018) statement are insufficiently science-based. Some of them appear to be less than scientific and rather more political; they may even be considered biased and populist.

Most worrying is that SAM uses an array of arguments that are also repeatedly used by various other stakeholders and proponents of deregulation of GMOs derived from new methods of genetic engineering. SAM wrongly claims that:

- no distinction can be made between the 'naturalness' of genetic engineering compared to conventional breeding;
- precision means safety; therefore, genetic engineering is less risky than conventional breeding;
- all genetically modified organisms should be treated equally;
- organisms derived from new methods of genetic engineering cannot be detected.

We were, in fact, surprised that the SAM statement, which supposedly represents the highest possible standards of scientific expertise, was thoroughly lacking in sufficiently rigorous scrutiny. If this statement is presented to political decision-makers, it is likely to convey a completely false impression and lead to erroneous conclusions.

In the following sections, we present some of our findings to illustrate the need for major revision of the SAM statement.

Genetic engineering and the concept of 'naturalness'

SAM states that "*In the light of current scientific knowledge, it is worth reflecting whether the concept of 'naturalness' is useful when deciding on regulatory requirements for organisms with an altered genome.*"[1]

As statements from leading researchers, such as Jennifer Doudna (Doudna & Sternberg, 2018) and George Church (Church & Regis, 2012), which talk of a 'crack in creation' and 'the end of the beginning', show, the criterion of 'naturalness' is still applicable and also crucial.

[1] For ease of reading, we have deleted references originally integrated in the quotes

Jennifer Doudna, in her recent book “A Crack in Creation” (2017) explains, the new methods of genetic engineering, and especially the CRISPR technology, can be used to bring the natural processes of evolution, that have emerged over nearly four billion years, to an end: “*Gone are the days when life was shaped exclusively by the plodding forces of evolution. We are standing on the cusp of a new area, one in which we will have primary authority over life’s makeup and all its vibrant and varied outputs. Indeed, we are already supplanting the deaf, dumb, and blind system that has shaped genetic material on our planet for eons and replacing it with a conscious, intentional system of human-directed evolution.*” (Page 243/244).

Similarly, George Church, another leading expert in the field of genome editing, in his book “Regenesis” (2012) states: *„Synthetic genomics has the potential to recapitulate the course of natural genomic evolution, with the difference that the course of synthetic genomics will be under our own conscious deliberation and control instead of being directed by the blind and opportunistic processes of natural selection.”*

It has to be emphasised, that, besides genetically engineered organisms, so far, all organisms can be considered ‘natural offspring’ of the ‘first cell’ and are not technically designed by mankind: Natural mechanisms, such as gene regulation and patterns of reproduction, still work and matter, whether or not the organisms are domesticated. There are some suggestions that we believe must be carefully considered; these imply that the “nature of life” should be legally safeguarded for the future as a protected common good (Chapron et al., 2019).

Conventional breeding is ‘not natural’?

SAM also refers to recent scientific findings showing an increase in knowledge regarding spontaneous mutations occurring in the genome: “*From the time of the adoption of the GMO Directive until now, owing to progress in analytical methods, extensive scientific evidence has been accumulated on spontaneously occurring genetic alterations”*

“These include point mutations (changes within a single letter in the genomic DNA), insertions, deletions and rearrangements of the genome, as well as the acquisition of exogenous genetic material across species or even kingdoms.”

It is true that a lot of data have been generated on spontaneously occurring genetic alterations. However, at the same time, new findings show there are many differences between spontaneous or induced genetic modifications and the pattern of genetic changes due to genetic engineering.

These findings include natural mechanisms of gene regulation that can impact, direct or even control spontaneous or induced genetic modification by naturally occurring mechanisms in the cells (for overview, see Kawall, 2019). In short, modern biology tells us that changes in the genome do occur spontaneously, and in summary, these processes are neither predictable nor completely random.

Conventional breeding, when using spontaneous or induced mutation, does not escape these natural mechanisms. In short, the methods and mechanisms used in what is known as 'conventional' breeding:

- make use of genetic diversity as a starting point;
- are applied to the whole cell or organisms;
- do not insert genetic information using direct technical interventions;
- do not delete genetic information using direct technical interventions.

However, genetic engineering can either partially or completely circumvent many natural mechanisms and controls developed over the course of evolution. Consequently, the resulting biological characteristics and associated risks can be profoundly different compared to those obtained from conventional breeding. Therefore, the current EU GMO regulation is justified and necessary to distinguish methods of genetic engineering from those of conventional methods of breeding.

How to distinguish 'nature' from 'human intervention'?

We agree with SAM (2018) that 'naturalness' can be helpful to identify GE technologies which need to be regulated according EU GMO regulation: "*Therefore, if referred to in the legislation, the concept of 'naturalness' should be based on current scientific evidence of what indeed occurs naturally, without any human intervention, in organisms and in their DNA.*"

However, we think that human intervention should be sufficiently qualified in this context, to not mix technical intervention with processes for selection, cultivation or crossing and selection: There is no proof that new biological characteristics can be obtained by escaping the natural processes when conventional breeding methods are used.

Therefore, in this context, human intervention should be interpreted as directly intervening at the level of the genome, i.e. inserting material that was prepared outside of the cells to achieve targeted changes in the genome or epigenome. This definition is in line with the definition of Directive 2001/18, encompassing those organisms that must undergo a mandatory approval process.

Precision and safety

SAM gives the false impression that conventional breeding is unsafe and plants derived from genetic engineering should be considered safe because of a higher level of precision; it starts with a statement on so-called random mutagenesis: "*Random mutagenesis, which has been used extensively in plant breeding since the 1960s, alters an organism's genome at multiple positions in a non-targeted way by treatment with a chemical mutagen or irradiation.*"

It is true that thousands of varieties have been bred from methods of conventional mutagenesis. Therefore, it seems that the methods used may, to some extent, be considered as showing a history of safe use. In general, mutagenesis may be thought of as speeding up the processes of evolution, but not escaping the mechanisms of evolution. As mentioned, there is no proof that by using conventional methods for breeding, new biological characteristics can be obtained by escaping the natural processes.

Ultimately, breeding through mutagenesis creates greater genetic diversity, but the desired traits are not brought about by direct technical interventions. It is only through crossing and selection of plants and animals exhibiting desired traits that a new variety can emerge from biodiversity. This process is time-consuming and requires careful choice and repeated testing by breeders. Nevertheless, some specific organisms (products) resulting from conventional breeding might require risk assessment in regard to health and the environment.

SAM mostly addresses the quantity of changes as introduced by random mutagenesis; this is presented as a reason for concern since it causes many unintended changes in the genome: "*Changes introduced by random mutagenesis are usually more drastic than those resulting from gene editing techniques, and include not only numerous point mutations, but also deletions and major rearrangements of genome fragments. (…) the ultimately selected end products are likely to*

carry additional mutations beyond the ones resulting in the desired trait, each of which can be considered to be an 'unintended effect'."

However, since conventional breeding always starts with a broad range of genetic diversity followed by further steps of crossing and selection, additional mutations cannot be considered to be 'unintended' but rather as 'intended'. A high rate of mutation is desired in plants because it contributes to greater genetic diversity and is necessary for following steps in conventional breeding, i.e. crossing and selection. Therefore, mutations might by classified as favourable or unfavourable, but not as 'unintended'.

Contrary to conventional breeding, genetic engineering is not based on, or aiming to, use a large pool of genetic diversity. Rather, the goals of technical intervention are targeted changes in the genome. Therefore, only under these circumstances can the term 'unintended effect' be used in meaningful way, as in the current GMO Regulation. It seems the SAM statement intentionally tries to confuse this context and correct meaning.

"Gene editing techniques can produce specific alterations at precise locations in the genome ranging from point mutations through to the targeted deletion or insertion of a gene, of parts of a gene or of other functional DNA sequences. Because of their precision, these gene editing techniques produce fewer unintended effects than random mutagenesis techniques. In addition, the end product is better characterised with respect to specific mutation(s) in the targeted position(s)

Because unintended effects will occur less frequently in gene edited products, these products are potentially safer than the products of random mutagenesis."

Since the category "*unintended effects*" can hardly be applied in this context in regard to conventional breeding, this comparison is not based on 'sound science'. In general, greater precision has nothing to do with greater safety or with higher success rates in breeding. Imprecise modifications, such as those resulting from conventional mutagenesis, can be both safe and beneficial. For example, the desired traits derived from conventional breeding are in many cases so-called quantitative traits loci (QTLs) which reveal the trait only in combination. The single components of the QTLs cannot in many cases be easily defined and remain unknown. The possibility of using QTLs is a huge advantage in conventional breeding compared to genetic engineering, which mostly works with 'building bricks' of defined genetic information in isolation.

Most relevant differences between genetic engineering and conventional breeding do not concern the quantity of changes, but rather the quality and specific patterns of genetic change. Due to the methods used in genetic engineering, the resulting patterns of genetic change as well as biological characteristics and associated risks can be substantially different compared to those derived from conventional breeding. For example, the application of CRISPR/Cas on wheat (Sanchez-Leon et al., 2018), enabled the targeted change of up to 35 copies within one specific group of genes. This is different to the results of conventional mutation breeding. Furthermore, so-called multiplexing might be applied, which means that not just one, but several gene families will be affected (Shen, L. et al., 2017).

Another example showing that even very tiny changes of just a few nucleotides in specific combination can trigger major biological effects and associated risks is the so-called 'Monarch-Fly': A gene in fruit flies (Drosophila melanogaster) was adjusted to a similar gene in the monarch butterfly. Just three tiny changes in individual base pairs within a gene can make the fruit flies resistant to toxins produced by specific plants. As a consequence, the flies ingest the toxin and thereby become toxic to other animals feeding on them. Releasing the flies into the environment may have detrimental effects on the food web and interconnected ecosystems (Karageorgi et al.,

2019). If such genetically engineered organisms are not strictly regulated, they might be released unnoticed into the environment.

To decide whether such organisms are safe or even "*safer than the products of random mutagenesis*", detailed examination of their genetic and overall biological characteristics is needed. Thus, there is a need for regulation as foreseen by current EU GMO regulation, even if no additional DNA sequences are inserted.

Similar products, but not treated equally?

SAM proposes that all genetically modified organisms, no matter whether they are genetically engineered or derived from conventional breeding, should only be assessed in regard to the intended characteristics of the product. This would mean setting aside current regulation which requires that all organisms derived from methods of genetic engineering (such as introducing either genetic material or material that enacts a change to genetic material into the cell) have to undergo mandatory risk assessment: *"the features of the final product itself must be examined regardless of the underlying technique used to generate that product."*

"From the above it follows that the regulatory framework for GMOs should put much more emphasis on the features of the end product, rather than on the production technique. As long as this is not the case, situations can arise where two products are identical, but because of different methods used in their production, they would have to meet completely different regulatory requirements."

"...the safety of a product is determined by its characteristics and not by the way it was generated. Therefore, the impossibility of distinguishing between spontaneously occurring mutations and different types of human interventions is a major issue from a regulatory point of view."

It should be remembered that, in its definitions, EU GMO regulation requests that new technical methods for directly intervening in the genome, known as 'genetic engineering' have to undergo an approval process. On the other hand, EU regulation and the term 'GMO' also encompass methods used in conventional breeding that are exempt from any mandatory approval processes.

In addition, as mentioned above, there are further good reasons to distinguish between genetic engineering (in a strict sense) and other methods of genetic modification (broader meaning): In many cases, the processes of genetic engineering and genome editing involve methods, such as 'gene canon' or agrobacterium, which can be combined with the usage of nucleases, often applied in multiplexing or serial applications. These steps in the technical process can coincide with specific unintended and intended changes that are different compared to those observed in other plants (Eckerstorfer et al 2019).

Consequently, processes used in genetic engineering can cause biological effects of a different biological quality, engendering new risks compared to those resulting from conventional breeding (including conventional mutagenesis). As a result, plants which seem to have similar traits might still be very different in their overall biological characteristics and risks because of the technology that was used to engineer them.

Scientifically, it seems to be impossible to assess the safety of the GE organisms without taking data from the process into account. For example, bacterial DNA was found to have been unintentionally (Norris et al., 2019) in the genome of genetically engineered cattle (SDN3 via TALENS). Data from the process are essential to detect such sequences. Thus, current EU GMO Regulation correctly

requests that all organisms derived from processes of genetic engineering generally require specific, case-by-case risk assessment.

Detectability

SAM (2018) suggests that organisms derived from the new methods of genetic engineering will enter the market without the possibility of identification. Stating their position, the experts refer to the example of non-browning mushrooms that are deregulated in the US: *"In addition, the obligations, imposed by the GMO Directive, on traceability and labelling of GMOs entering the European market will be very difficult to implement and control due to issues related to the detection, identification and quantification of gene edited products (…) . This will become more difficult when exporting countries start to market varieties that they have already decided not to regulate. An example is the case of gene edited mushrooms developed to have a reduced tendency to brown."*

The application of nucleases, such as CRISPR Cas or TALENs, causes typical patterns of genetic change that can be used for identification and traceability (Duensing et al., 2018). This is also the case with the aforementioned mushrooms: In this case, several copies of one gene were changed to block the production of a specific enzyme (Waltz, 2016; Gartland et al., 2017). It is unlikely that a similar mushroom has ever existed on the market. The mushrooms should therefore be easily traceable if, within the approval process, the specific data on the specific pattern of introduced genetic changes are made available.

As far as the deregulation of products in the US is concerned (see Testbiotech, 2019), this finding seems to be applicable to all the GE organisms derived from methods of genome editing. It does, however, require the necessary data to be made available. More research might still be needed to quantify the concentration of a product in case of accidental contamination.

It should be taken into account that products derived from genetically engineered organisms already have to undergo an approval process and labelling; they have to be traceable even if a detection method is not available (such as in the case of oil from genetically engineered organisms).

Final findings and conclusions

There are several reasons why organisms derived from new methods of genetic engineering all have to undergo mandatory risk assessment:

In many cases, the old techniques of genetic engineering (such as agrobacterium and biolistic methods) are used in a first step (for overview see: Testbiotech 2019) to insert the nucleases (such as CRISPR/Cas, TALENS) into the cells. It is only in a second step that the nuclease (the enzyme) is produced by the cells and starts 'cutting' at the targeted site. It is known from current literature that these first steps give rise to many unintended effects, such as deletions and rearrangements that can include the unintended insertion of additional DNA, and impact gene expression (see, for example, Rang et al., 2005). It is also known that these methods can impact epigenetic regulation (Jupe et al., 2019).

The specific on-target and off-target effects of the application of the nucleases largely depend on technicalities such as (i) the specific nuclease used, (ii) the target organisms, (iii) the targeted genes, (iv) the way in which the nucleases are introduced into the cells, (v) the dosage of the enzyme and (vi) duration of the intervention (for overview, see Eckerstorfer et al., 2019; Agapito-Tenfen et al. 2019) These technical details need to be taken into account by the competent authorities in order to identify potential unintended effects caused specifically by the process of technical intervention.

The intended effects in many cases show specific patterns of genetic alteration because the nucleases in most cases will impact all (or at least many) copies of the target gene throughout the genome. For example, TALENs was used in in sugar cane to change 107 out of 109 gene copies of one gene to improve its quality for agro-fuel (Kannan et al., 2018). Furthermore, as mentioned, so-called multiplexing might be applied, which means that not just one, but several gene families will be affected (Shen, L. et al., 2017).

In short, the pattern of intended and unintended changes and the resulting new combinations of genetic information arising from genome editing will in most cases be different in comparison to those derived from conventional breeding. These differences co-occur with biological characteristics and new risks that need to be fully investigated before any conclusions on the safety of the new organisms can be drawn. Detailed examination of an organism's genetic and overall biological characteristics is needed to decide whether such organisms are safe.

This need for regulation as foreseen by current EU GMO regulation, is given, even if no additional DNA sequences are inserted. Very tiny targeted changes can have huge impacts on the overall biology of the organisms (see, for example, Karageorgi et al., 2019).

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