Testbiotech basic text 23-8-2023

Differences between new genetic engineering and conventional breeding (non-targeted mutagenesis)

There are some fundamental differences between new genetic engineering processes (New GE, also new genomic techniques, NGT) and non-targeted mutagenesis used in conventional plant breeding. These differences are important for the risk assessment and identification of the genetically engineered plants. We have, therefore, summarised some of the differences between New GE and non-targeted mutagenesis in the following section.

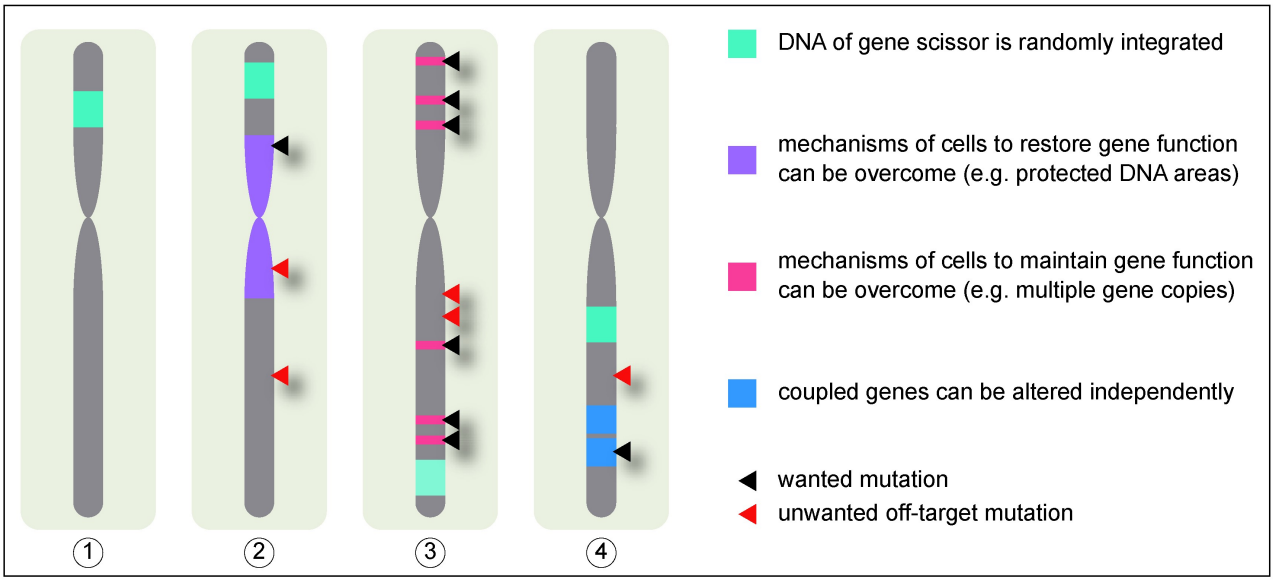

Figure: New genetic engineering applications in plants can result in genetic changes which are unlikely to occur with conventional breeding. One reason: unlike conventional breeding (including non-targeted mutagenesis), new genetic engineering can overcome the limitations of naturally evolved genome organisation. The figure shows NGT applications using CRISPR/Cas on 4 DNA samples (simplified). Example 1 shows the transgenic DNA needed to produce the CRISPR/Cas nuclease being randomly integrated in the plant genome. Example 2 shows an intended mutation and an unintended mutation, both induced in a protected region of the DNA near the centromeres, with a further unintended mutation occurring in another region. Example 3 shows how the gene scissors can alter several (in this case six) copies of the same gene simultaneously, which would be unlikely to happen with conventional breeding; other unintended mutations also occurred in this case. Example 4 shows genetic linkage; the gene scissors can alter linked genes independently of each other even though they are typically inherited only as a pair.

New GE in plants is typically used to achieve genetic changes that go beyond what can be obtained from conventional breeding. It does not require the insertion of additional genes. Unlike conventional breeding (including non-targeted mutagenesis), NGTs can overcome the constraints of natural genome organisation brought about by evolution, including maintenance mechanisms and/or

restoration of gene functions, e. g. repair processes, gene copies and genetic linkage. CRISPR/Cas ‘gene scissors’ in particular are able to make more extensive changes to the genome in comparison to conventional plant breeding, and thus enable what could be called a greater 'depth of intervention'.

In addition, New GE processes can also result in unintended DNA changes, which may differ in their patterns, sites and biological effects from those seen in conventional breeding. There are several reasons for this: in most cases, the transgenic DNA for the production of the gene scissors (CRISPR/Cas) is introduced into the genome using non-targeted methods. ‘Old’ genetic engineering methods are used for this purpose. These often cause unintended changes in the genome and the multiple insertion of DNA fragments, which often remain undetected. At the end of the process, so-called segregation breeding is applied to remove the transgenes from the plant genome, but nevertheless,unintended genetic changes will remain in many cases.

After the gene scissors are synthesized in the cells, they are meant to actively target specific genomic regions. As a result, in most cases, both strands of the DNA are cut. This step in the process may cause other unintended genetic changes, e. g. the confusion of target sequences. Another example are so-called ‘catastrophic events’ in the genome (chromothripsis), caused by the double strand breaks in the target regions. Therefore, while it is possible to use gene scissors to target particular sites in the genome, it is not possible to sufficiently predict and control the consequences of these interventions in regard to the genome, the plants or the environment.

If the plants are not examined in detail, the unintended genetic changes can persist in their offspring and accumulate in populations through subsequent crossing. Long-term risks to humans and the environment can also not be ruled out. Consequently, a detailed analysis and risk assessment is necessary before the safety of the plants can be evaluated. Further examples and sources can be found at: https://www.testbiotech.org/en/limits-to-biotech

Table: Differences between new genetic engineering and conventional breeding (non-targeted mutagenesis)

Criteria	Breeding / non-targeted mutagenesis	New genetic engineering
Purpose and precision	Non-targeted mutagenesis increases the range of genetic variants in the genome of plants in less time than is the case naturally. The increased genetic diversity is then the starting point for selection, followed by further crossings and selection during the breeding process.	New genetic engineering is not typically used to increase genetic diversity. On the contrary, it is intended to generate only very specific changes in the genome. In many cases, the goal is to achieve genotypes (genetic changes) and phenotypes (biological effects) that are unlikely to be obtained from conventional breeding. For this purpose, it is not necessary to introduce additional genes into the genome. Changing just a few building blocks of the DNA (e.g. far less than 20 nucleotides) can for example be sufficient to 'knock-out' individual genes. However, the gene scissors are not always precise and can also cause changes outside the target region. In addition, the desired double-strand breaks also cause many unintended changes in the target regions. Therefore, while it is possible to use gene scissors to target particular sites in the genome, it is not possible to sufficiently predict or control the consequences of these interventions in regard to the genome, the plants and the environment.
Genome organisation and epigenetics	The outcomes of non-targeted mutagenesis depend upon various factors, including cellular mechanisms protecting gene functions like repair mechanisms, additional gene copies, linked genes and others, such as epigenetic factors.	New genetic engineering and, in particular, the CRISPR/Cas gene scissors, can overcome natural mechanisms (see figure) with which cells protect certain gene functions. As a result, not only the processes, but also the outcomes (genotypes and phenotypes) can differ significantly from those of conventional plant breeding. Overall, the outcomes of new genetic engineering are not limited by natural mechanisms protecting gene functions to the same degree as is the case with conventional breeding.
Pattern of genetic changes	Plants in particular often have a redundant genome, i. e. genetic information is available in several copies. If many copies of a specific gene are present in the genome, these copies typically cannot be changed simultaneously using conventional breeding methods (and non-targeted mutagenesis). Therefore, the gene functions are preserved, but possibly restricted.	New GE procedures very often cause multiple genetic alterations: all gene copies with the same or similar sequence can be changed simultaneously. This includes the ability to target several different genes (and their gene copies) at the same time – a process known as 'multiplexing'. The resulting pattern of genetic alterations typically is specific to New GE processes, and very often cannot be achieved with conventional breeding methods. These patterns of genetic changes can also be used as a 'fingerprint' to identify New GE plants.

Criteria	Breeding / non-target mutagenesis	New genetic engineering
Repair mechanisms in the cells	In many cases, the cells are able to repair the damage caused by mutations and restore the original version of the gene.	If cell repair mechanisms restore a DNA segment altered by CRISPR/Cas to its original version, the nuclease is able to recognise its target region again and can remain active until the original structure is permanently changed. Thereby, a gene function can be 'knocked out' that otherwise would have been restored.
Number and sites of genetic changes	In non-targeted mutagenesis, the number of genetic changes can be higher than with new genetic engineering methods. The changes achieved typically do not go beyond what might be expected naturally. However, the changes do occur within a shorter period of time.	New genetic engineering is able to change specific sites in the genome, and thus generate new gene combinations that would not otherwise be expected using conventional breeding methods. The main decisive factor is not the number of mutations, but rather the sites and functions of the altered genes. Even small changes can have major effects that go far beyond the results of non-targeted mutagenesis.
Differences to natural plant populations	The phenotypes of cultivated plants often differ from the natural non-domesticated forms. This is due to the fact that naturally occurring characteristics are selected according to specific breeding goals, and the resulting traits can be much more pronounced than is the case in wild populations.	The resulting patterns of genetic changes (genotypes) and the biological effects (phenotypes) are often significantly different and more 'extreme' if compared to those in conventional breeding.
Speed of development	Unexpected new traits may also emerge naturally or with conventional breeding. Typically, these are rare events and thus allow enough time for the adaptation to the surrounding ecosystems.	New GE enables the release of many organisms that are not adapted to the environment within short periods of time, comprising many species. Similar to climate change, it is the speed of developments that can overstretch the resilience of natural systems.