FGU Project Genetic Engineering and the Environment

Factsheet: The potential of NGTs to overcome constraints in plant breeding and the regulatory implications

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November 2025

Summary:

A new publication investigates the differences between genetic changes in plants that occur in classical plant breeding and those induced using the tools of new genomic techniques (NGTs), e.g. CRISPR/Cas9 ‘gene scissors’ (Koller 2025).

In conventional plant breeding, several constraints are known that limit the outcome. The constraints are caused by plant biology and cellular mechanisms such as enhanced repair of certain genes, additional ‘back-up’ gene copies and combined inheritance of genes via genetic linkage. Other than in conventional breeding, these constraints are often just minor hurdles for recombinant enzymatic mutagens (REMs) such as CRISPR/Cas, also known as ‘gene scissors’.

The new publication shows that the outcomes of NGT applications can be very different to those of conventional breeding, including those when physico-chemical mutagens are applied. The reason for this is the specific mode of action: While physico-chemical mutagens such as radiation or chemicals merely cause breaks in the DNA, REMs such as CRISPR/Cas additionally interfere with the cellular repair mechanisms. More recently developed REMs even expand the capabilities of NGTs to introduce new genetic variations within the target sequences.

NGTs therefore allow the introduction of genetic changes and combinations that are unknown in the current breeding pool. These new genotypes are also unlikely to occur with any previously used breeding methods and may therefore need to be considered as ‘new to the environment’.

The debate about some of the differences has been ongoing for several years. However, the new publication results in new and strong evidence by systematically assessing recently published data. Detailed analysis was performed in regard to constraints in conventional breeding as well as the mode of action of REMs and the resulting NGT plants.

The differences are not only significant for expectations regarding innovation in plant breeding, but also for the risk assessment of plants obtained from REMs. NGT plants should therefore be subject to a process-based approval process and risk assessment that compares the outcomes with those obtained from conventional breeding on a case-by-case basis.

Introduction

Evolution has developed mechanisms and processes which allow species to adapt to changing environments and at the same time protects them from too many mutations within short periods of time. Conventional plant breeding takes place within the framework of these mechanisms and processes, which means that they present constraints for what can be achieved through breeding methods even when physico-chemical mutagens are applied.

In biotechnology, recombinant enzymes are engineered to efficiently catalyze specific reactions. Recombinant enzymes are also applied in plants to introduce genetic changes in the genome as e.g. 'gene scissors' such as CRISPR/Cas. They can be referred to **r**ecombinant **e**nzymatic **m**utagenes (REMs) which can produce outcomes that cannot be obtained from previously applied methods.

The sections below summarize the main findings of Koller (2025). They give an overview of constraints in plant breeding (Section 1), show how REMs can overcome these constraints (Section 2) and discuss the regulatory implications of these findings (Section 3).

1. Constraints in conventional plant breeding

Evolution has developed cellular mechanisms and processes that constrain the outcomes in conventional plant breeding. The constraints in conventional plant breeding can be categorized into the following groups:

(1) Cytogenic features:

Organisms are permanently exposed to DNA damage. In many cases, the cell repairs the damage and restores the original gene function. However, the repair mechanisms are not equally active or effective throughout the genome which means that in some genomic region, spontaneous and non-targeted mutations occur less frequently than in others.

(2) Factors influencing recombination (**Figure 1-1 & 1-2**):

During sexual reproduction (and the process of meiosis), maternal and paternal genomes are mixed and recombined by 'crossing over', resulting in new genetic combinations. Usually, the number of crossover events is kept at a low level, occurring preferentially at narrow hotspots. This results in groups of genes, that tend to be inherited together (genetic linkage). Naturally occurring chromosomal rearrangements, larger inversions and deletions also seem to occur non-randomly at hotspots.

(3) Gene copies (**Figure 1-3**):

Plants have significantly more gene copies than most other eukaryotes due to increased gene duplication rates. Using conventional plant breeding techniques, it is difficult, time-consuming and in part impossible to modify or knockout all the different copies of a gene. Further constraints exist, if the gene copies lie close together and are genetically linked, as in gene clusters.

(4) Other factors that may need further research:

Further research may be needed to analyse further factors, elements and mechanisms, such as the microstructure of regulatory units, that are known to limit the outcomes of conventional plant breeding.

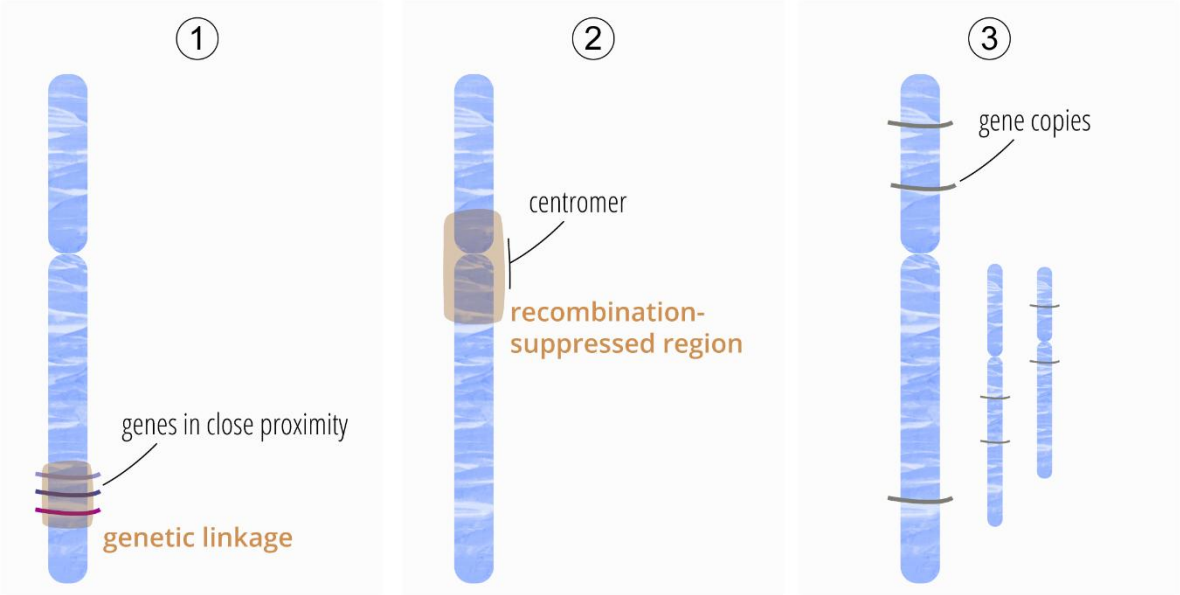

Figure 1: Constraints in conventional plant breeding: (1) genetic linkage, (2) recombination-suppressed regions and (3) gene copies.

2. Recombinant enzymatic mutagens (REMs) can overcome constraints of conventional breeding

Many experiments have shown that CRISPR/Cas9 and other REMs can overcome constraints in plant breeding. For example, its nuclease can interfere, delay or substantially hamper cellular repair mechanisms (**Figure 2**). It can also induce double strand breaks, deletions and inversions in genomic regions where otherwise mutations or recombinations are rarely observed. Finally, the nuclease can change or introduce gene combinations that may not be achievable with conventional breeding.

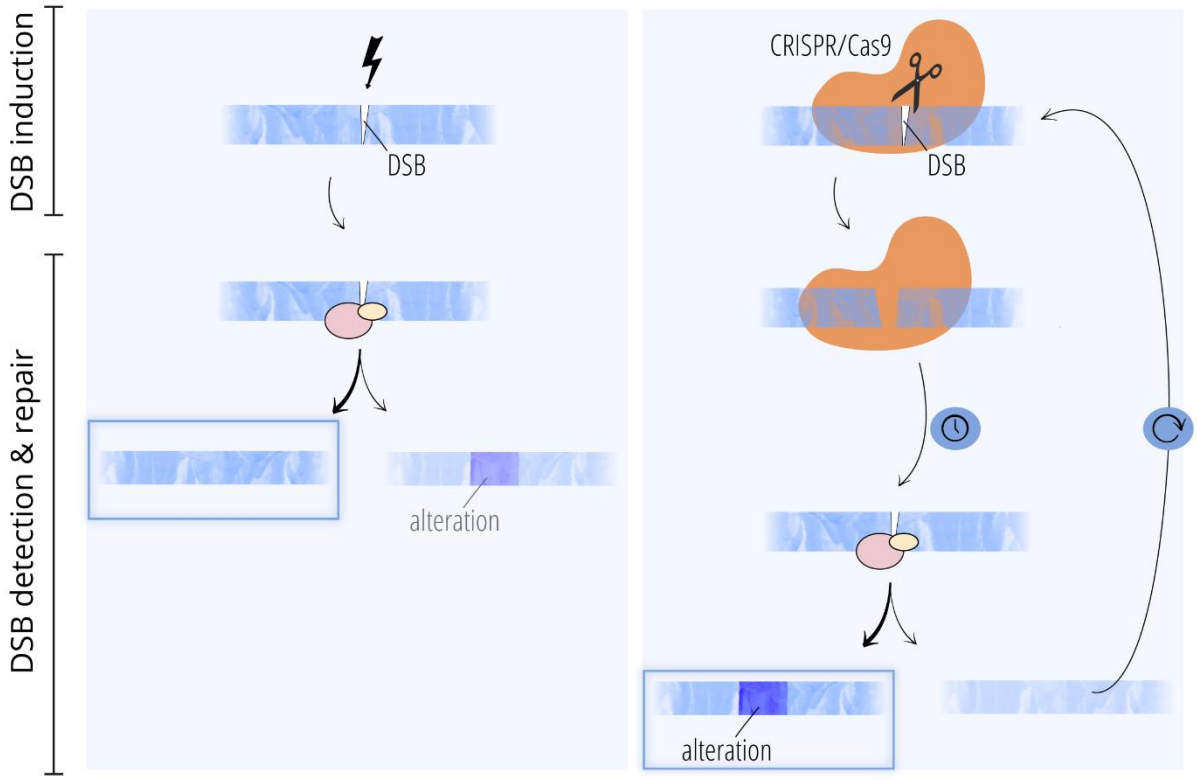

Figure 2: Repair of double strand breaks (DSBs) without (left) and with (right) recombinant enzymatic mutagens (REMs). Left: The DSB that occurs spontaneously, or is induced in a non-targeted way by physico-chemical stressors, is detected and repaired by repair proteins (red and yellow circle). Either the previous state is restored or the sequence is altered. Right: After the induction of a DSB, CRISPR/Cas9 stays bound to the cleaved ends until the enzyme is, e.g. dislodged from the DNA. Here, the DSB detection, processing and repair is therefore delayed compared to otherwise induced DSBs. If the previous state of the DNA sequence is restored via a DNA repair process, the enzyme CRISPR/Cas will detect it and catalyze the reaction again and ultimately force a change. Left and right: The most likely outcomes are framed.

A number of publications and examples have demonstrated, that the outcome of NGT applications in plants can be very different compared to conventional breeding methods, e.g. plant species such as camelina, maize, mustard, poplar, rice, sugar cane, switch-grass, tomato and wheat. The examples include different traits including changes in plant composition, fastening of breeding processes, early first flowering, changes in interactions with soil bacteria, *de novo* domestication, altered plant architecture and drought resistance. In most cases, these new plant characteristics are achieved via minor genetic changes, an overall small number of mutations, and without the insertion of additional DNA.

More recently, large databases allow the use of specific AI programs to identify the target regions and generate the design for the most effective genetic changes, e.g. in regulatory units. These applications often require several targeted changes within short distances, which would be hardly achievable using conventional breeding or even the original CRISPR/Cas9 enzymes. More recently, scientists have developed advanced REMs, which allow specific deletions within target sites of the genome (CRISPR/Cas12a), specific changes of key nucleotides (base editors) or the introduction of small artificial DNA sequences (prime editors) (**Figure 3**).

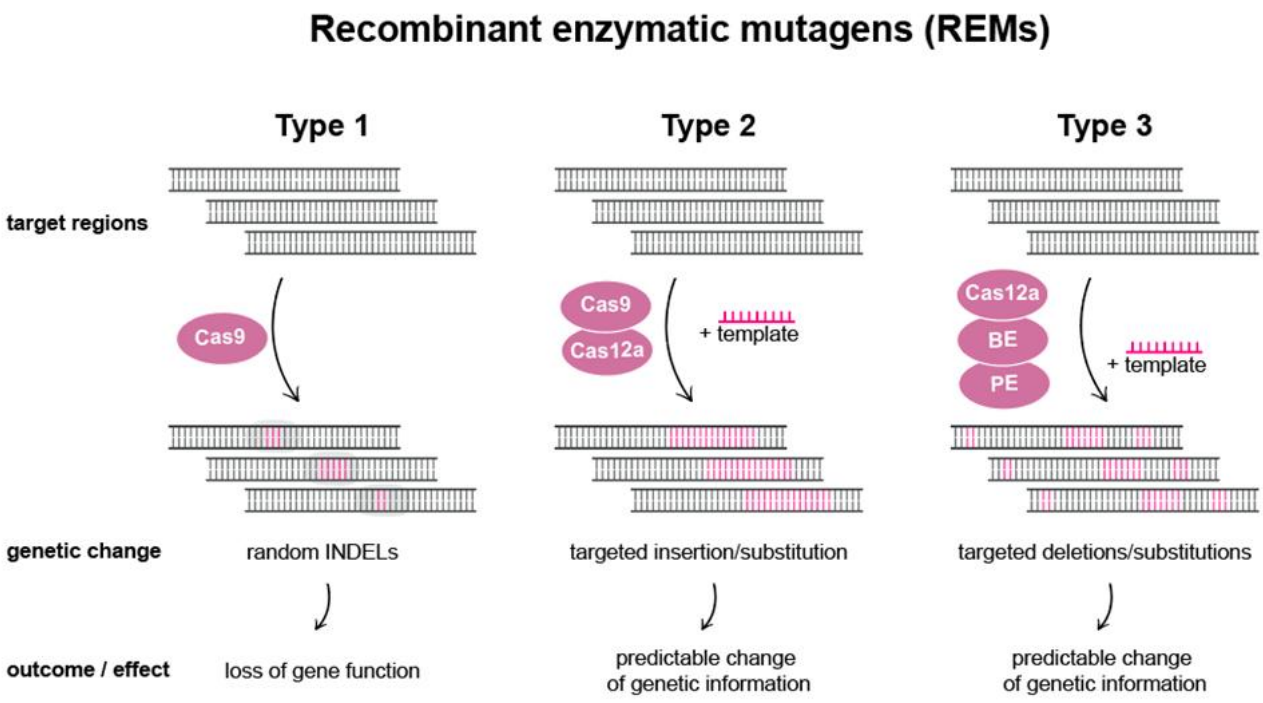

Figure 3: Application of recombinant enzymatic mutagens (REMs) leading to different outcomes. The application of REMs can be categorized into three different types. Type 1 (left): REMs like CRISPR/Cas9 induce double strand breaks in the target region. Repair of Cas9-induced double strand breaks results primarily in random small insertions and deletions (INDELs). Most often, this leads to the loss of previous gene functions. Type 2 (middle): REMs like CRISPR/Cas9 or /Cas12a can be used to introduce external DNA by using templates. Type 3 (right): CRISPR/Cas12a, base editors (BE) or prime editors (PE) can further expand the capabilities of NGTs to introduce new genetic variations within the target sequences, e.g. specific deletions or exchange of key nucleotides. Type 2 and Type 3 lead to more predictable changes of the genetic information.

Overall, the publication provides compelling evidence that that different causes of mutations can lead to different outcomes, also providing a long list of examples for respective NGT plants.

3. Regulatory impacts

The above findings are decisive for expectations regarding innovation in plant breeding. They are also relevant for the regulation of NGT plants, as the new plant genotypes, their intended and unintended genetic changes as well as their resulting effects may be associated with health and environmental risks.

Recombinant enzymatic mutagens (REMs) make it possible to intervene in the genetic properties of organisms to a much higher degree than previously conceivable. They make the plant genome available for genetic changes that are otherwise highly unlikely to occur. As a result, REMs can be used to produce plants with characteristics that go way beyond the known characteristics of the respective species, even if only small genetic changes are induced and no additional genes are inserted. REMs can be applied in many plant species, which means that a wide range of diverse NGT traits and organisms may be released into the environment in huge quantities and in a short time.

EU plans for future regulation of NGT plants do not take into account the differences between the application of REMs and the processes and outcomes of conventional plant breeding. This

observation is relevant to CRISPR/Cas9 enzymes as well as to more recently developed REMs that further expand the capabilities of genome editing. AI now allows scientists to rapidly search large databases to identify and to generate specific genetic changes especially in regulatory units. This opens up for a larger design-room for previously unknown gene variations, particularly in combination with the new types of REMs. Therefore, amount and complexity of those 'fine-tuned' NGT plants can be expected to increase in future. Thus, making them highly relevant to the future regulation of NGT plants.

However, the current proposals for the future EU regulation of NGT plants did not reflect on these finding and more recent developments. There are numerous examples of NGT plants with novel characteristics, that would not, according to the current proposal, have to undergo risk assessment. Well argued risk scenarios can be developed or are already available for several NGT plants showing that environmental risk assessment is necessary before any releases take place.

These examples show that the technical potential of REMs must be taken into account in regulatory provisions. Otherwise, previously unknown genotypes and phenotypes may negatively impact plant health, ecosystems, biodiversity and plant breeding. It must further be acknowledged that the different outcomes of NGTs and conventional breeding are not always evident at first sight. As a starting point, molecular characterization can inform the following steps in risk assessment and guide requests for further data.

5. Conclusions

NGT outcomes can be very different to the processes involved in previously applied breeding methods, including physico-chemical mutagenesis. The reason for this is the specific mode of action: While physico-chemical mutagens such as radiation or chemicals merely cause a break in the DNA, recombinant enzymatic mutagens, such as CRISPR/Cas, additionally interfere with the cellular repair mechanisms. If the substrate (i.e. the target DNA) is restored via DNA repair, the CRISPR/Cas enzyme will repeatedly catalyze the reaction and ultimately force a change. More recently developed REMs even expand the capabilities of NGTs to introduce novel genetic changes going beyond what is known from conventional breeding as well as of CRISPR/Cas9 applications.

Consequently, NGTs allow the introduction of genetic changes and combinations unknown in the current breeding pool; these are also unlikely to occur from the application of any previously used breeding methods. The new genotypes may ultimately need to be considered as 'new to the environment'. CRISPR/Cas catalyzed reactions can in particular interfere with and overcome 1) cytogenic features such as repair mechanisms, 2) factors influencing recombination and stability of genome such as crossovers and 3) gene copies with and without proximity.

The findings are relevant to the regulation of NGT plants, as the new plant genotypes, their intended and unintended genetic changes as well as their effects may be associated with higher health and environmental risks.

Reference

Koller F. (2025) The potential of NGTs to overcome constraints in plant breeding and the regulatory implications. https://doi.org/10.20944/preprints202511.0397.v1

Abbreviations

AI	artificial intelligence
CRISPR/Cas	clustered regularly interspaced short palindromic repeat/CRISPR-associated
NGTs	new genomic techniques
REMs	recombinant enzymatic mutagens