New genome editing techniques and variety evaluation

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Abstract

In 2007, a working group set up by the European Commission identified a set of eight plant breeding techniques (or "N(P)BT" according to the acronym "New (Plant) Breeding Techniques"). Among these NBTs, directed nuclease techniques, including the CRISPR/Cas technique, are known as genome editing techniques. They allow fine and precise modification of genomes by creating modifications in the nucleotide sequence of the DNA at predetermined and chosen targets.

The European Commission has announced a policy action on plants derived from targeted mutagenesis and cisgenesis in 2021. The aim would be to adapt the authorization and risk assessment procedures as well as the traceability and labelling requirements, while maintaining a high level of protection for health and environment.

In this context, the French Ministry in charge of Agriculture asked in November 2021 the Scientific Committee of the CTPS (Permanent Technical Committee on Plant Breeding) to shed light, on the basis of the scientific and technical literature, on the impact of the evolution of genome editing techniques on the evaluation of varieties.

Evolution of NBTs and genome editing techniques and impact on varietal panel

There have been strong developments in the use of the CRISPR system since 2016, when the last report of the CTPS Scientific Committee on the subject was published, both in the technique itself and in the methods for introducing CRISPR/Cas into plants.

The first noticeable evolution is the expansion of variability in PAMs motifs, which allows to operate on a wider range of targeted sequences on the DNA. Base editing and prime editing methods allow to induce targeted and selected modifications of one or more bases at the same time. The real evolution is the ability of these methods to "choose the base(s)" in a precise way, without using random modifications of the DNA. The use of an "inactive" Cas protein makes it possible to enhance its precise binding action in the genome, as an effector recruiting platform. It thus gives the possibility to act on gene expression. This method also makes it possible to act on the epigenome by modifying epigenetic marks. The multiplex modification method allows to target several DNA sequences simultaneously. This allows the modification of several targets in a single action, and thus the possibility to act on regulatory pathways, to increase the number of edited traits or to act on the expression of several genes or multi-gene families. Among the future developments, the CRISPR system makes it possible to work on chromosomic rearrangement, by inducing chromosome inversion or translocation.

Several methods consisting in using the CRISPR system in a ribonucleoprotein form have been developed, making it possible to avoid the use of a plasmid coding for the CRISPR/Cas9 complex, and thus to reduce the number of off-target mutations, thanks to its short-lived action. To overcome the constraints linked to recalcitrant species and the methodological complexity of in vitro culture, two methods were developed, de novo meristem induction and "Virus-induced heritable gene editing" consisting in using viral DNA and its capacity for systemic and directed diffusion of the complex in the plant. Currently, however, the techniques used in varietal creation require a transgenic step with the insertion of an exogenous DNA fragment.

To date, there does not seem to be any major revolution among edited materials commercialised outside the European Union. However, more innovative approaches are being studied, particularly in relation to regulation mechanisms. Genome editing techniques can help to make the main selection targets more accessible (which are also worked on with other breeding methods: post-crossing recombination, non-target mutagenesis, transgenesis, etc.). The main strategy is to obtain, by directed mutagenesis, the effects obtained by other mutation methods, most of the modifications currently proposed being of the "knockout" type, i.e., inhibiting the expression of a gene. The traits studied and obtained by genome editing are still of simple determinism, but the perspectives of innovation offered by multiplexing in relation to more complex traits could bring new solutions to current challenges. The applications of this method still need to be fine-tuned, but the range of possibilities it offers suggests real advances in plant breeding.

Evaluation of varieties obtained from NBT

The new breeding techniques are promising tools, whose full potential on the varietal offer is not yet known. Currently, these tools have only developed a small range of possible applications in plant breeding. The possibility of having more complex traits accessible to targeted modifications suggests changes in responses to biotic and abiotic stresses. For traits and metabolic pathways that benefit from in-depth scientific knowledge, the prospect of acceleration of breeding schemes in agricultural sectors makes them an interesting tool to respond more quickly to agronomic, societal, industrial, and ecological expectations. The services and disservices provided by agronomic traits that are within an already existing variability within the species, and that could be obtained by different selection techniques, are not specific to the use of NBTs. NBTs offer an interesting potential to contribute to agricultural sustainability through accelerated innovation and more accessible traits. However, the widespread use of NBTs could potentially lead to the rapid deployment of a trait across a territory, with a possible reduction in the number of species cultivated because they have been edited, and lead to risks linked to the homogenization of certain traits (erosion of resistances, vulnerability of the ecosystem, etc.). NBTs also allow the creation of new variability that can show new effects, never obtained by conventional breeding, for example a very high protein level, the release of highly expressed secondary metabolites, or a strong resistance to a pathogen. These disruptive innovations, which can provide solutions to major constraints, may on the other hand be associated with disservices that need to be evaluated. Finally, " gene-centred " approaches, often used to solve targeted problems, are generally not in phase with the necessary improvement of a production system and the systemic and multi-character evaluation of innovations for agroecological transition. They must be combined with each other and with other levers to be relevant. And to be combined, the proposed editions will have to remain compatible with varietal selection. Major modifications of chromosome structures or cell functions could indeed make crosses difficult/impossible with the resources worked on in parallel in varietal selection. Given the importance for variety breeding of the use of newly registered varieties in crosses, such genetic incompatibilities could constitute biological barriers to the breeder's exemption.

The use of genome editing techniques does not challenge the major principles of variety evaluation for registration. If the product is the object of the control, the CTPS is the most adapted framework for the evaluation of the traits of the varieties according to the services they provide to the users, and it has already largely demonstrated its capacity to integrate new claims and traits. However, the evaluation of the risks of potential disservices, as deployed for GMOs, is not covered by the CTPS and should be considered in the case of disruptive modifications. Thus, it will be necessary to make a distinction between the edited traits: a trait similar or close to what can be obtained by conventional breeding, or a new trait that is highly modifying, to adapt the applicable rules. In the case of the obtention of disruptive and potentially impactful traits, it will be necessary to characterize the services that could be provided and to be explicit about the disservices that may arise. It will also be necessary to consider the impacts of new traits on the crop and its environment, and to study the possible release of disruptive traits into the environment (risk of a large-scale transfer). If the technique is subject to specific rules, it will be necessary to set up procedures for prior evaluation, traceability, and monitoring after the registration of varieties.

Impact of the release of NBT varieties

The market access of varieties with traits derived from NBT techniques will result in the coexistence of NBT and non-NBT varieties on the market. Moreover, unlike GMOs, there is no straightforward solution to detect varieties with edited traits, the identification of which would rely on a laborious and costly process (sequencing and involvement of all stakeholders, in the absence of specific detection methods). This would hamper the monitoring of these editions in the cultivated compartment and the detection of leakage in the wild compartment. This difficulty adds to those already present with GMOs and makes the coexistence of the chains very difficult. To ensure coexistence between edited and non-edited varieties, the notion of traceability is crucial. If "NBT-free" chains are set up, the cost of certification of these chains will be a question to be addressed as a priority. There is a risk of crystallising certain opposing positions, with a risk of certain types of varieties disappearing. The societal acceptability of a food offer partly derived from NBT products must be clearly considered. It is important in the discussions and considerations to underline the difference between edited varieties and GMO varieties, in a factual way. The release of NBT varieties may also have impacts on plant genetic resources. NBT methods give access to an infinite number of alleles and could lead to a looser management of plant genetic resources, or to abandon their conservation. Vigilance will be required on this point, as the use of these resources in conventional breeding has contributed to the resilience of agriculture up to now. In this context, it is essential to better characterize these resources, for a better use but also to have a comparison with the claimed edited traits, and to preserve them.

Intellectual Property of varieties derived from NBTs can be covered by plant variety rights (PVR) on the varieties, patents on the methods used to obtain the varieties and patents on the edited traits. The development of genome editing techniques, which are largely patented and licensed, raises questions of access to the technique. The access cost is important, leading to difficulties of access for certain species, sectors, and economic operators. This could result in a concentration of research efforts on certain species and varietal types/traits, in contradiction with the diversification needed for agroecology. Furthermore, independently of the new genome editing techniques, it is worth reminding that the granting of patents on native alleles discovered in genetic resource accessions is a reality, even if the number is low. However, in this picture of IP titles, the development of NBTs and patents on edited traits will lead to enhanced intellectual property issues, as it will potentially lead to an increase in patented elements in commercialized varieties. To facilitate the work of breeders and, in line with the above-mentioned traceability needs, access to information on the intellectual property attached to commercialized varieties, and regarding edited traits, is an important issue. Yet a greater occurrence of patented elements in varieties could lead to more obstacles to the inclusion of these varieties in breeding programs, with royalties to be paid or with the need to suppress patented alleles in the derived varieties.

To conclude, between 2016 and 2022, major technological advances have been developed in genome editing techniques. These advances have enabled fine-tuned editing of a greater diversity of molecular targets, and therefore of traits. Although many traits have been published, at this stage these are mostly proofs of concept, on a limited number of agricultural species, with little practical impact. It is assumed that there is a real perspective of speeding up the process, if economic and social considerations do not hinder it. The use of genome editing techniques does not challenge the main principles of variety evaluation for registration, as the CTPS is able to adapt to the evaluation of new traits. For edited traits corresponding to naturally variable characteristics and already studied for registration, it does not seem necessary to modify the procedures. However, for disruptive innovations that result in new traits, an evaluation should be made to characterize both services and disservices, at a time and space scale that is appropriate for the trait claimed to be improved, its use, and its compatibility with other breeding methods. If future regulations impose a particular control according to the breeding technique used, it will be necessary to set up an evaluation, traceability, and monitoring procedure after the registration of varieties. To enable the coexistence of edited and non-edited varieties, the notion of traceability is essential. The issue of the social acceptability of edited plants must be clearly addressed and considered. This must lead to reflection on the traits for which editing would allow a benefit widely shared by the different stakeholders. Another assurance of acceptability may be the guarantee that minor species, likely to contribute to diversification, benefit from similar research and breeding efforts, including these new technologies. The issue of intellectual property is very important in the discussion on NBTs. In addition to the tools that allow intellectual property to be granted to genetic material based on its phenotypic characterization (PVR), and to an inventive industrial technology (patent on CRISPR/Cas), there is also the possibility of patenting edited genes, which is already used. Patents on living organisms are one of the causes of the societal non-acceptability of certain plant breeding techniques. Within the Scientific Committee of the CTPS, different points of view have been expressed on this subject (i) there is no difficulty in allowing patents on traits and PVR to coexist; ii) it is recommended not to patent edited traits), with no consensus being reached.