Position statement of ENSSER (European Network of Scientists for Social and Environmental Responsibility) on the CRISPR/Cas gene editing technique in agriculture and horticulture

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The CRISPR/Cas system is a commonly used technique to undertake gene editing. Gene editing is indisputably an artificial laboratory-based method of genetic modification that gives rise to genetically modified organisms (GMOs), as confirmed by the 2018 ruling of the European Court of Justice (European Court of Justice, 2018).

CRISPR/Cas has captured the imagination of scientists, the media, and the public. It is claimed to be capable of making intensive agriculture more sustainable, reducing pesticide use, producing disease- and pest-resistant crops, improving the nutritional value of crops, making crops more tolerant to environmental stresses such as drought, and preventing livestock animal diseases.

However, CRISPR/Cas is simply a method of targeting a genetic modification (disruption of a gene, alteration of a gene's protein coding sequence or insertion of a gene or other genetic element) to a predetermined location in the genome. As detailed below, there is no evidence that crops and livestock animals developed using CRISPR/Cas and other gene editing techniques will fulfil the promises being made for them. On the contrary, they pose risks that must be acknowledged, studied, and controlled through robust regulation.

Not precise

It is the ability to target the "edit" to a specific sequence in the genome that prompts claims of precision and predictable outcomes for CRISPR/Cas. However, the edit takes place after the CRISPR/Cas has performed its function, which in most cases is the generation of a double-strand DNA break. The edit results from the subsequent activation of DNA repair processes, which are prone to errors. Thus the outcome of the edit is neither precise nor fully under the control of the genetic engineer, as it is at the mercy of the cell's own DNA repair processes.

As a result, as well as the intended genetic modification, CRISPR/Cas gene editing can result in unintended mutations (DNA damage), both at the intended edit site ("on-target") and elsewhere in the genome ("off-target"). These unintended mutations can include large deletions, insertions, and rearrangements of DNA, the creation of novel gene sequences resulting in mutant protein production, unintended modifications at locations in the genome similar to the target site, and chromothripsis (a destructive genomic rearrangement that results from the shattering of individual chromosomes and subsequent haphazard rejoining of the pieces), which give rise to legitimate safety concerns (Chu and Agapito-Tenfen, 2022) (Kawall, 2021) (Kawall et al., 2020) (Eckerstorfer et al., 2019).

In the case of gene-edited plants (whether obtained via CRISPR/Cas or other new genetic modification methods), unintended effects of the gene editing process as a whole (tissue culture, cell transformation, unintended/intended outcomes from the gene editing tool) will inevitably result in unwanted altered patterns of gene function. These in turn can change plant biochemistry in such a way that it unexpectedly produces novel toxins or allergens, altered levels of existing toxins or allergens, or altered nutritional value. Such changes could have negative impacts on the health of human or animal consumers and on the wider ecosystem, as scientists have warned (Eckerstorfer et al., 2019) (Kawall et al., 2020) (Kawall, 2021) (Agapito-Tenfen et al., 2018).

In the case of gene-edited animals, unintended changes have reportedly led to abnormalities such as enlarged tongues, extra vertebrae, and premature death (Rana and Craymer, 2018). Gene-edited cattle were unexpectedly found by US FDA scientists to contain bacterial DNA which included gene sequences conferring resistance to three different antibiotics (Norris et al., 2020), in spite of the developer's claim that the cattle were free from unintended effects (Carlson et al., 2016) and the company's and allies' insistence that they did not need to be regulated at all (Carroll et al., 2016)(Regalado, 2019).

While these cattle were gene edited using the TALEN rather than the CRISPR/Cas tool, the same outcome could have occurred from CRISPR/Cas gene editing, as the error occurred during the repair process following the creation of a double strand DNA break. The repair process, governed by the cell's repair mechanisms, unintendedly incorporated the entire bacterial plasmid DNA repair template.

Mutations from gene editing are different from those that arise in nature

The mutations caused by gene editing processes are different in quality from those that arise through natural reproduction and from chemical- and radiation-induced mutagenesis breeding. In natural reproduction, certain regions of the genome are protected from mutations through various mechanisms, including heightened recruitment of DNA repair machinery, whereas gene editing makes the whole genome accessible to mutations (Kawall, 2019).

Furthermore, genetic variations that arise through rounds of natural reproduction are not random. It has been discovered that in natural reproduction of Arabidopsis plants, genetic variations arise not randomly but in a directed inheritance manner that is “biased” towards those that benefit the plant (Monroe et al., 2022). In contrast, gene editing is designed to override natural protections against mutations to enable mutations to occur that would be impossible or extremely difficult to achieve by natural breeding (Kawall, 2019). Thus, arguments that the unintended mutations that occur during plant gene editing can be ignored as a risk, since random genetic variation takes place during natural breeding, are incorrect. The non-random, biased directed genetic variation that occurs from natural reproduction is far less likely to lead to deleterious gene expression outcomes than are the random genome-wide mutations resulting from the gene editing process as a whole.

In addition, it must be recognised that risk increases with scale. Inducing intended and unintended mutations throughout the genome of a plant through gene editing and releasing it at large scale in many locations, as occurs with commercial crop plants, is different in magnitude from mutations arising in nature or from conventional breeding. Consequently the risk is also greater and demands robust regulation (Heinemann et al., 2021).

Foreign DNA

One argument that is often used to advocate for deregulation of gene editing is the claim that techniques such as CRISPR/Cas do not result in the insertion of foreign DNA into the genome of the organism. However, this is false. GM gene-edited plants and animals can and do contain foreign genetic material in their genomes, either by intention or inadvertently, due to the inherent imprecision of the gene editing process.

Intentional insertion of foreign genes or DNA occurs in so-called SDN-3 (gene insertion) applications of gene editing.

Unintentional insertion of foreign bacterial plasmid (circular) DNA molecules can also occur in gene editing. This is because most CRISPR/Cas plant gene editing is carried out via the transgenic GM technique of introducing plasmids into plant cells. The plasmid encodes for the gene editing tool (the protein and guide RNA elements of the CRISPR/Cas complex). Once inside the plant cells, these genes are expressed, resulting in assembly of the CRISPR/Cas editing tool, which then carries out the edit. However, the plasmid introduced into the plant cells can fragment, with these fragments being randomly inserted into the plant cell genome (Kim and Kim, 2016). Each one of the randomly inserted plasmid DNA fragments constitutes an insertional mutagenesis event that can interrupt the normal functioning of one or more genes. In addition, even if the inserted fragments of plasmid DNA do not encode for a protein, they may still be expressed into RNA, which can exert gene regulatory functions resulting in disturbed gene expression patterns.

Foreign plasmid DNA can also inadvertently integrate into the genome of gene-edited animals, through a different mechanism than in plants (Norris et al., 2020) (see “Not precise”, above, for the example of the gene-edited cattle).

In addition, foreign bacterial chromosomal DNA from Agrobacterium has been found to inadvertently integrate into the genome during the genetic modification process. Agrobacterium infection is used in older transgenic genetic modification methods. It is the most efficient genetic modification transformation technique for plants, so it continues to be the most frequently used technique to introduce plasmid DNA encoding gene editing tools into plant cells including CRISPR/Cas.

It has been found that DNA fragments from Agrobacterium of up to 18,000 base units in length – large enough to contain whole genes – can integrate into the plant genome during the genetic transformation process (Ülker et al., 2008). As Agrobacterium infection is commonly used in gene editing, functional whole genes of Agrobacterium could be introduced into plants during the gene editing process.

In the case of gene-edited animals, foreign DNA from animal-derived culture media can inadvertently be inserted into the genome (Ono et al., 2019) (Latham, 2019).

The implications for health and the environment of the presence of foreign DNA or genes in gene-edited plants and animals – as well as the implications for the plants and animals themselves – are as yet unknown.

However, the risks of gene editing techniques such as CRISPR/Cas are not restricted to the presence of foreign genes or DNA, but apply equally to intentionally simple applications of gene editing (so-called SDN-1, or “gene disruption” applications) (Kawall, 2021). Even if it is established that foreign genes are not present in the final marketed product, unintended mutations will still accumulate from the different stages and components of the gene editing process: tissue culture, the GM transformation process, and gene editing tool activity. These can lead to the risks to health and the environment described above.

Regulatory requirements to protect health and environment

As gene editing has inherent unpredictable and unintended genetic and functional consequences, in order to minimise the risks from these procedures, all products of CRISPR/Cas must be subjected to robust regulation that is both process- and product-based. It is knowledge of the process that provides insight into the mechanisms of how things can unexpectedly go wrong, as well as the intended gene edit.

This means that regulators must consider all the processes used to develop the gene-edited plant or animal (tissue culture, cell transformation, and action of the gene-editing tool), since unintended mutations can accumulate from each stage (Tang et al., 2018), with consequent risks of unintended effects (Chu and Agapito-Tenfen, 2022) – as well as the intended trait. Any attempt to exclude consideration of the gene editing process and

focus solely on the product is not being true to the science underpinning this technology and puts at risk public health and the environment.

Regulations must require that long-read whole genome sequencing is performed in pre-market testing, as short-read sequencing, which is used in the vast majority of studies on plant gene editing, can easily miss unintended mutations (Kawall et al., 2020).

In addition, molecular compositional profiling methods ("omics" methods: gene expression-profiling transcriptomics, protein-profiling proteomics, and small biochemical molecule-profiling metabolomics) must be carried out, to ascertain if a gene-edited plant or animal is equivalent to its non-gene-edited parent except for the intended gene editing outcome.

Molecular profiling methods are used by research groups across the world to gain insight into the functioning of living organisms. The computational tools (bioinformatics and statistical methods) used to analyse the data from these methods are well established and enable scientists to interpret the data and their implications for health and environmental safety. Thus there is no excuse to exclude molecular profiling methods from regulatory procedures governing gene editing of crops and livestock animals. Indeed, regulators would be negligent if they did not require molecular profiling to be included in the risk assessment of gene-edited products, as only such methods can provide the necessary first step in assessing safety.

Detection is possible

It is often argued that there is no point in regulating gene editing because detection of gene-edited products is impossible. However, any GMO (including those produced with gene editing) can be detected in the laboratory, provided the developer makes available information on the genetic changes made and provides regulators with reference samples of the GMO. The reference material allows the placement of a gene editing event (even if it is just a one DNA base unit change) within the unique genomic context of the plant or animal and allows unequivocal determination of its presence. In addition, scientists based at EU Member State regulatory agencies have called for "international coordination to set up an appropriate state-of-the-art database" of gene-edited food and feed products in various countries to assist detection (Ribarits et al., 2021). We agree with this proposal as an essential first step to preserve traceability and facilitate labelling for the consumer.

Sustainability and gene editing

Advocates of deregulation for gene-edited GMOs often argue that the urgency of the sustainability and climate crises demand that these GMOs are quickly brought to market without the delays and expense caused by the current GMO regulations. However, there is no evidence that gene editing can contribute to solving these problems.

For example, a study published in 2022 investigated crop plants developed with "new genomic techniques", as described in existing studies and field trial reports, to identify to

what extent their intended traits were able to fulfil the EU's sustainable development goals. The researchers primarily focused on the desirable traits of drought tolerance and resistance to fungal pathogens.

The researchers found that there are no available plants developed using new GM techniques with drought tolerance traits and that overall, no GM technique (older or new) had made a positive contribution to drought tolerance (Hüdig et al., 2022). Documentation on pathogen resistance was largely restricted to proof-of-principle studies and reports of successes at laboratory or greenhouse stages, with only two publications on field trials. Few genes have been identified as conferring resistance to multiple pathogens, a prerequisite for avoiding broad-spectrum fungicide use in conventional agriculture.

The researchers concluded that plants developed with new genomic techniques that can withstand more than one stressor or different environments “are not documented in advanced development states”. The researchers concluded that plants developed with new genomic techniques will not be sufficient to achieve the EU’s sustainability goals and that a variety of agricultural measures “will need comparable attention and research efforts” to those currently applied to new GM plants (Hüdig et al., 2022).

These conclusions are not surprising, given that desirable characteristics such as drought tolerance and pathogen resistance are complex genetic traits, meaning that they are conferred by the functioning of multiple gene families acting as a coordinated network. Such complex traits cannot be conferred by manipulating one or a few genes by gene editing. In contrast, conventional breeding, which is able to bring together the gene families that underpin complex traits, has been successful in achieving these traits in many different crops (Nowell, 2022) (Gilbert, 2014)(Lakhani, 2022) (Filmer, 2020)(Chouraqui and Chowdhury, 2020).

A separate review notes that plant gene editing and older GM techniques share the qualities of imprecision and causing unpredictable outcomes. In the case of older GM techniques, these outcomes have affected agricultural performance and environmental sustainability. Therefore there can be no shortcuts when regulating new GM techniques. The review concludes, “Despite the promise of new traits and techniques, GM crops, including gene-edited crops, are unlikely to meet either the narrow agronomic, or the broader social and environmental requirements of sustainable agriculture.” The review ends with a discussion of how plant breeders can best support and promote sustainable agriculture, and thus help create sustainable food systems (Wilson, 2021).

Yet another review looks at the history of older-style GM crops in Africa and asks whether gene-edited crops can benefit crop breeding, based on claims of precision, cheapness and speed. The authors challenge each of the claims, pointing to mounting evidence that gene editing is not precise. Neither, they state, is it likely to be cheap, given the dominance of CRISPR patents by Corteva Agriscience, which will lead to a further “concentration of corporate control similar to that which constrained the release of GM technology”. They also question the claim of speed. They conclude that there is a need to “move beyond the

genome” to “prioritize the co-development of technologies with farmers, seek out non-patented material and acknowledge that seeds are a single component of highly complex agroecological and production systems” (Rock et al., 2023).

Conclusion

It is evident that gene editing procedures, including the use of CRISPR/Cas, through different mechanisms introduce a wide spectrum of unintended mutations within the genome of the target organism. These large-scale unintended mutations will be associated with altered patterns of gene function, which could lead to changes in biochemistry and production of novel toxins and allergens. Furthermore, outcomes from the application of gene editing in an agricultural context especially with respect to crops, have been poorly characterised since they are lacking in molecular compositional profiling and appropriate toxicity testing. In the absence of such empirical evidence, it is incorrect to depict CRISPR/Cas gene editing as precise and predictable and to claim that its food products are safe.

Based on the inherent imprecision of the gene editing procedure and the detailed reasons given above, gene-edited products, including those produced using CRISPR/Cas, must remain regulated under current EU GMO regulations. Risk assessment, traceability requirements, and on-package GMO labelling must be maintained, just as for currently available GM products.

Furthermore, the risk assessment guidelines must be tightened to ensure that all unintended changes resulting from the gene editing process, as well as the intended changes, are evaluated for safety, health and the environment.

Claims made for gene editing techniques – of precision, predictability, safety and effectiveness in solving agronomic problems – must be based on evidence. This is particularly needed since CRISPR/Cas gene editing is in its infancy and has no history of safe and effective use in agriculture.

Therefore those who advocate for the deregulation of gene editing techniques and their products are at odds with scientific and agronomic reality. The evidence shows that the way forward in meeting future challenges in food and agriculture is to promote systemic solutions, such as agroecological farming, and, where needed, produce new crops and animal breeds through the proven effective method of conventional breeding, augmented where needed by the safe biotechnology of marker assisted selection.

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