2. Gene editing is not precise and causes unpredictable genetic errors

MYTH

Gene-editing tools such as CRISPR/Cas bring about changes in the genome in a precise and controlled way, with predictable outcomes.

REALITY

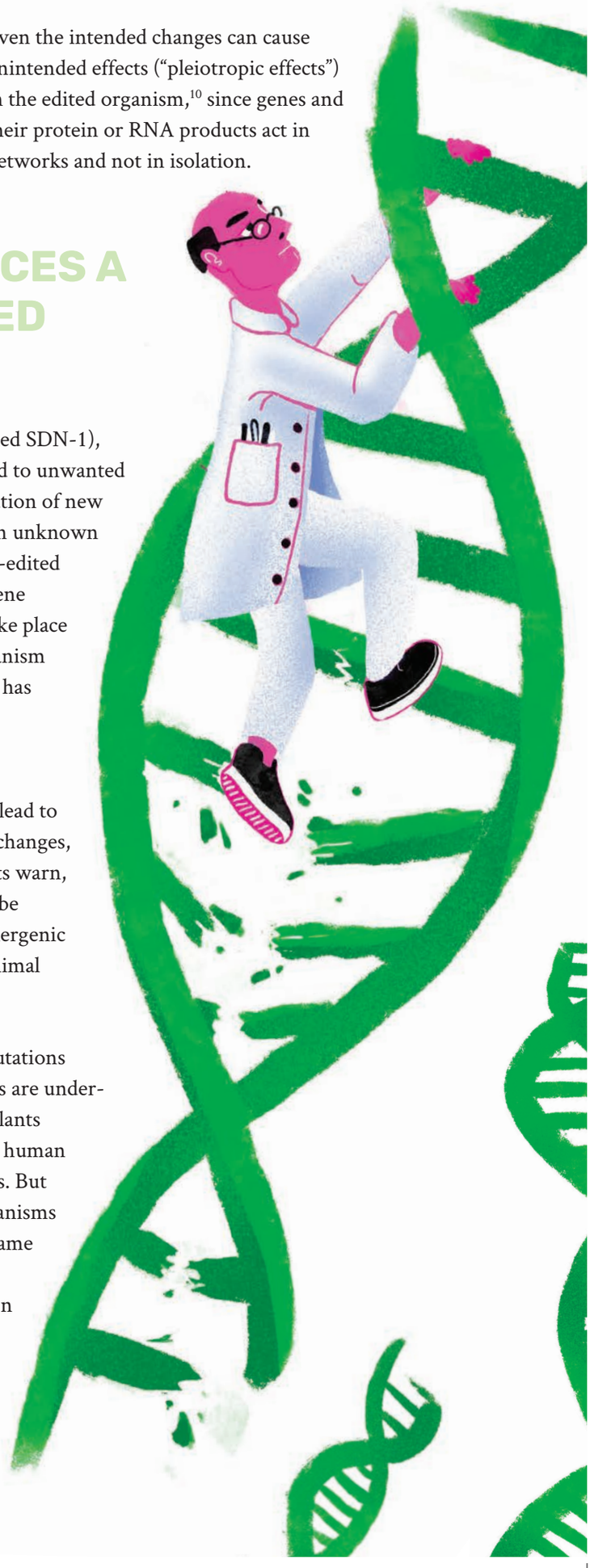
Gene editing is not precise, but causes many genetic errors, with unpredictable results, in addition to any intended genetic change.

The agricultural biotech industry and its allies claim that gene-editing tools such as CRISPR/Cas bring about changes in the genome in a precise and controlled way.[1,2,3] Some even claim that they bring about only the specific intended changes and nothing else.[4,5] They argue that gene-edited products should therefore be excluded from the regulatory oversight applied to older-style transgenic GMOs,[3,5] where (in most cases) DNA is introduced from another species into a part of the genome that cannot be determined beforehand.

However, these claims do not survive scrutiny.A large and ever-growing number of scientific studies in human, animal and plant cells show that gene editing is not precise but gives rise to numerous genetic errors, also known as unintended mutations (DNA damage). These occur at both off-target sites in the genome (locations other than that targeted for the edit) and on-target (at the desired editing site). The types of mutation include large deletions, insertions, and rearrangements of DNA.[6,7,8]

These mutations occur at various stages of the process, including stages that gene editing has in common with old-style transgenic GM methods, such as tissue culture and GM transformation by Agrobacterium tumefaciens infection (in which this soil bacterium is used to insert the foreign genetic material into the DNA of plant cells).[9]

Even the intended changes can cause unintended effects ("pleiotropic effects") in the edited organism,[10] since genes and their protein or RNA products act in networks and not in isolation.



GENE EDITING PRODUCES A RANGE OF UNINTENDED MUTATIONS

Even the simplest application of gene editing (so-called SDN-1), which is intended to destroy a gene function, can lead to unwanted mutations.[11,12,13] These mutations can lead to the creation of new gene sequences producing new mutant proteins, with unknown consequences to the health of consumers of the gene-edited organism. In addition, alterations in the pattern of gene function can take place within the organism whose genome has been modified.

> Unwanted mutations can lead to the creation of new gene sequences producing new mutant proteins, with unknown consequences to the health of consumers of the gene-edited organism

In plants, these alterations can lead to compositional changes, which, scientists warn, could prove to be toxic and/or allergenic to human or animal consumers.[6,8,14]

Unintended mutations and their effects are under-researched in plants compared with human and animal cells. But since the mechanisms of gene editing and subsequent DNA repair are the same between animals and plants, there is every reason to believe that the types of unintended mutations seen in human and animal cells will also be found in plants. Recent research in rice plants attests to this fact.[15]

A study on rice varieties found that CRISPR gene editing caused a wide range of undesirable and unintended on-target and off-target mutations. The researchers were aiming to improve the yield of already high-performing varieties of rice by disrupting the function of a specific gene, in an SDN-1 (gene disruption) procedure.[15] They were trying to produce small insertions and deletions of DNA base units in the genome. However, what they got was quite different. In many cases they found large insertions, deletions, and rearrangements of DNA, raising the possibility that the function of genes other than the one targeted could have been altered.[15]

As for the hoped-for increased yield, the opposite was found – yield was reduced.[15] This should not come as a surprise, as yield is a genetically complex trait that involves the functioning of many, if not all, gene families of the plant. Thus altering the function of one gene to improve yield could be viewed as a futile exercise.

> In plants, alterations in the pattern of gene function can lead to compositional changes, which could prove to be toxic and/or allergenic to human or animal consumers

The researchers warned that CRISPR gene editing "may be not as precise as expected in rice". They added, "early and accurate molecular characterization and screening must be carried out for generations before transitioning of CRISPR/Cas9 system from lab to field".[15] Developers do not generally do this, or if they do, the results are not published.

The researchers concluded, "Understanding of uncertainties and risks regarding genome editing is necessary and critical before a new global policy for the new biotechnology is established".[15]

INADEQUATE SCREENING FOR UNINTENDED MUTATIONS

Most studies that look for unintended mutations in gene-edited plants grossly underestimate the number of mutations resulting from gene editing and associated processes such as tissue culture (growth of plant tissues or cells in a growth medium). This is true both for studies that conclude that gene editing causes many such mutations and those that conclude that it causes few or none. The reason is that the authors of these studies use inadequate detection methods – short-range PCR and short-read DNA sequencing – to look for mutations. They only look at short stretches of the DNA around the targeted editing site and computer programme-predicted off-target sites.

As Kosicki and colleagues found in a study on human cells, short-range PCR and short-read DNA sequencing can miss major genetic errors, such as large deletions and insertions

and complex rearrangements of DNA.[16,17] The researchers concluded that a combination of long-range PCR and long-read DNA sequencing is needed to spot the full range of unintended mutational effects.[16] FDA scientists have made the same recommendation, with regard to gene-edited animals.[18]

This principle applies to plants just as much as animals, since the mechanisms of gene editing and the subsequent repair that forms the "edit" are the same.

In a scientific review, Kawall and colleagues confirmed that the "vast majority" of studies on gene-edited plants used biased detection methods to screen for genetic errors, meaning that they will miss many such errors. Among studies on gene-edited animals, none included a thorough analysis of genetic errors.[6]

CIBUS'S CANOLA: "PRECISION" GENE EDITING OR ACCIDENT IN A PETRI DISH?

In September 2020, the biotech company Cibus claimed that its herbicide-tolerant SU Canola (oilseed rape) was not gene-edited but was the result of random mutation caused by tissue culture – effectively, an accident in a laboratory Petri dish. This claim came after the company had for many years said (including to regulators) that SU Canola was made with its "precision gene editing" technique, called oligo-directed mutagenesis (ODM).[19,20,21] In fact, ODM constitutes the very foundation of its business model.[22]

> The vast majority of studies on gene-edited plants used biased detection methods to screen for genetic errors

Indeed, numerous public records point to the fact that Cibus used gene editing in the process of engineering SU Canola.[19,20,23] But it turned out that the oligonucleotide used was designed to produce a different genetic change from the one that was found to confer herbicide tolerance in SU Canola and that Cibus described in its patent application.[21] So the "precision" tool did not work as intended, leading Cibus to announce that the crop was not gene-edited after all.

It would appear that Cibus made that claim only to evade EU GMO regulations. The timing is remarkable: Shortly before Cibus made its statement,[20] a scientific paper had been published, reporting the development of the first publicly available detection method for SU Canola.[24] However, under EU law, even if the specific mutation that confers the herbicide tolerance was not the intended result of the ODM editing process, the fact that the ODM tool was used to develop the SU Canola means that it is a GMO. Since it has no EU authorisation, its presence in EU imports would be illegal.[23]

This episode raises questions about Cibus's honesty and transparency. But more importantly, it shows that the precision and control claimed for the ODM gene-editing technique was false.

"OLD" MUTAGENIC GM TECHNIQUES ARE USED IN GENE EDITING

First-generation genetic engineering techniques are still often used to introduce CRISPR editing tools into plant cells. Plasmids containing genes encoding the CRISPR/Cas editing tool are introduced into the cells using either Agrobacterium tumefaciens infection or particle bombardment.[6] In addition, tissue culture is used to grow the plant cells. All three processes are highly mutagenic.[25] The mutations caused by these processes will be in addition to the unwanted mutations caused by the gene repair process (the actual "edit").

A study by Tang and colleagues on CRISPR gene-edited rice illustrates the mutagenic nature of these processes. The study found that many off-target mutations resulted from the tissue culture, and yet more resulted from Agrobacterium infection (around 200 per plant). In contrast, seed saved from non-GM rice plants had only 30–50 spontaneous mutations per plant.[9] Thus the study found that the CRISPR process, taken as a whole, caused large numbers of off-target mutations and far more than conventional breeding.

Ironically, this study is often cited as an example of the precision of this gene-editing tool. This is because it found that the CRISPR editing tools themselves did not introduce many off-target mutations into the plants' DNA.[9] However, this finding is likely not accurate, due to the researchers' use of inadequate screening methods (see "Inadequate screening for unintended mutations", above) – they did not use long-read DNA sequencing. Also, the findings must be viewed in the context of the above-mentioned separate study on rice that found that CRISPR gene editing caused a wide range of unintended on-target and off-target mutations.[15]

> A study on CRISPR gene-edited rice has found that many off-target mutations resulted from tissue culture, and yet more resulted from Agrobacterium infection

THREAT TO HEALTH AND ENVIRONMENT

Based on the above evidence, gene editing is neither precise nor controllable, but could inadvertently produce traits that threaten public health and the environment.

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