



Detecting a point mutation does not clarify its origin

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Does a point mutation look different when it is made by one process or another? No! One cannot tell from the mutation itself whether it was spontaneous or triggered by genome editing, and additional information on the history of the genetic material is needed as a precondition to evaluate from which breeding process it originates. Spontaneous or edited, point mutations are the same for all intents and purposes.

EPSO fully agrees that known gene edits including single nucleotide changes can be detected by PCR. EPSO declared this in its input to the present EC study on NGTs (New Genomic Techniques) and connected statements. The Greenpeace-funded work by the Chhalliyil et al (2020) publication merely confirms this well-established fact.

However, the published method has two main limitations: It does not present a means to establish that genome editing is the cause of the detected mutation, since it just displays a sequence modification without identification of the modification process. This has been seen from the beginning as the major challenge, since edited plants produced in countries with more open regulation are not declared as such. In addition, the method is not applicable to unknown gene modifications, since edited plants, contrary to classical GMOs (Genetically Modified Organisms), do not share common elements, and a method detecting a specific sequence variation cannot detect different variations in other plants and sequences. The detection of a single nucleotide change does not provide any proof by itself that this change was provoked by genome editing rather than natural mutation.

On 7 September 2020, Chhalliyil et al. published the paper 'A real-time quantitative PCR (Polymerase Chain Reaction) method specific for detection and quantification of the first commercialised genome-edited plant'. This is based on the detection of SNPs (Single Nucleotide Polymorphism) in two respective genes conferring a resistance to sulfonylurea and imidazolinone herbicides. One gene is thought to be modified by genome editing (ODM), the other by chemical mutagenesis. The authors developed a method to specifically detect these SNPs in the relevant sequences.

Chhalliyil et al. 2020 claimed that "certified seed from three SU (sulfonylurea and imidazolinone herbicide-tolerant) canola varieties, C1511, C5507, and 40K (Cibus US LLC/Falco Brand, San Diego, CA, USA) all developed by oligonucleotide directed mutagenesis [1,30] were used." and furthermore that "based on the fact that variety 5715 was the only Cibus ODM variety authorized/deregulated at the time varieties C1511, C5507 and 40K were commercialized, it can be concluded that these varieties were derived from variety 5715."

However, it is not clear that the mutation was in fact a product of genome editing (ODM): Health Canada (reference 30 in Chhalliyil et al. 2020) explained: "***The petitioner hypothesized that the single nucleotide mutation was the result of a spontaneous somaclonal variation*** that

occurred during the tissue culture process, and **not due to the specific oligonucleotide used in the RTDS protocol**. Moreover, Cibus describes: “MEET FALCO™, BROUGHT TO YOU BY CIBUS™ Falco™ sulfonylurea-tolerant (SU Canola™) canola is a first generation Cibus trait **developed through traditional plant breeding methods**. 32K, 68K and 40K SU Canola™ hybrids offer high yields and excellent weed control and will soon be joined by a number of other innovative hybrids as our product pipeline continues to grow.”

So Chhalliyil et al. (2020) describe a method to detect a SNP by qPCR, not a method to determine the origin of this mutation. The link to genome editing is not established by the method, but simply circumstantial: based on their inconsistent historic (pedigree) information on how the SNP was generated. They cannot judge by their method whether “natural” or “engineered”. In fact, the paper by Chhalliyil et al. 2020 underlines how important a priori information is, and that it is impossible to judge just from a short sequence whether it has been modified by genome editing or natural mutation. They actually demonstrate that the pure method is insufficient to judge compliance and scrutinize law enforcement.

Thus the conclusion in the EPSO statement “On the EC study on New Genomic Techniques (NGTs) *Brussels, 27.5.2020* is confirmed: “With regard to incoming NGT-plants or NGT-products, GMO legislation can readily be applied if the genome modifications are known, which is the case, for example, in international scientific collaborations. However, in the absence of prior knowledge on the potential genome alterations their detection and identification does not seem to be feasible by PCR-based detection methods. Often suggested as the ultimate tool, whole genome DNA sequencing actually allows under certain conditions the near-exhaustive detection of unknown DNA modifications in a plant genome. However, the detection of a sequence alteration does not permit the identification of the process that generated it and to decide whether GMO legislation needs to be applied or not. Indeed, identical DNA alteration may be obtained by NGTs or by conventional breeding or random mutagenesis techniques, which are exempted from GMO legislation.”

This statement was developed by Alan Schulman (EPSO President) and Peter Rogowsky (EPSO Agricultural Technology Working Group co-chair) and approved by the EPSO Board, based on previous statements approved by the Agricultural Technology Working Group and the EPSO Representatives.

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Useful links

- Chhalliyil et al. (2020) A real-time quantitative PCR method specific for detection and quantification of the first commercialised genome-edited plant. *Foods* 9:1245 [doi: 10.3390/foods9091245].
- EFSA draft scientific opinion on the safety assessments of plants developed using Site-Directed Nucleases type 3, SDN-3
https://www.efsa.europa.eu/sites/default/files/consultation/consultation/Scientific_opinion_SDN1_2_ODM_for_PC.pdf
- EC study on new genomic techniques (NGTs)
https://ec.europa.eu/food/plant/gmo/modern_biotech/new-genomic-techniques_en
- Court of Justice of the EU: Judgment in Case C-528/16, 25.7.2018. [English Press Release](#); [Ruling in English](#):

<https://epsoweb.org>

EPSO [Working Group on Agricultural Technologies](#):

Statements drafted by this group and approved by the EPSO representatives are for instance:

- [EPSO statement on the EFSA draft opinion on directed mutagenesis](#), 25.6.2020
- EPSO: [Statement on the EC study on New Genomic Techniques \(NGTs\)](#), 27.5.2020
- EPSO: [Statement on the Court of Justice of the EU ruling regarding mutagenesis and the GMO Directive](#), 19.2.2019
- EPSO: [EPSO welcomes Commissioner Andriukaitis statement and call for action 'New plant breeding techniques need new regulatory framework'](#), 29.3.2019

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