GMO Status of Cibus SU Canola

9 November 2020

Summary

- An organism is a GMO under EU law if a regulated genetic modification (GM) technique was used in its development, and the development process led to a genetic modification that does not occur naturally.
- It is well documented that oligonucleotide-directed mutagenesis (ODM), was used in the development of SU Canola. ODM is a regulated GM technique, according to the 2018 ruling by the European Court of Justice (ECJ).
- The EU's GMO laws were determined to be an appropriate precautionary response to manage the risks associated with novel genetic modification techniques, and an appropriate political response to consumer demand for informed choice. The ECJ has left no doubt about the laws' applicability to gene-edited organisms.
- Cibus, however, is trying to evade these laws. It is asking European regulators to accept the account that although ODM was used, an entirely unrelated, random event during tissue culture was responsible for achieving the targeted herbicide tolerance.
- Even if Cibus' account of how it arrived at SU Canola were correct, SU Canola would still be a GMO because it is not material under EU law whether the regulated GMO technique gave rise to the intended change or not.
- In any case, the company's own data indicate that SU Canola is likely the result of off-target effects linked to the application of ODM.
- The Commission and EU member states should now be implementing measures to ensure this unauthorised GM product does not enter the EU's food supply. This includes initiating a detection regime to screen for SU Canola, relying on the best method available.
- Prompt action is required as SU Canola is the first commercial gene-edited crop and so a test case for responding under EU law. To accept Cibus' narrative would send a signal to GM developers that they can evade regulation by creative interpretation of the law, and so undermine regulatory and legal authority.

Background

In September, an open-source method to detect a gene-edited, herbicide-resistant canola developed by US company Cibus, was published in the scientific journal *Foods*.[1] SU Canola is not authorised for placing on the market in the European Union.

Cibus' subsequent statements that the SU Canola varieties currently on the market in North America are "not gene-edited"[2] have caused some uncertainty about their GMO status, amplified by the European seed industry association's adoption of that narrative.[3]

This briefing sets out why SU Canola is a regulated GMO under the EU's GMO laws, and why EU competent authorities must ensure this GMO is not present in EU supply chains.

Contents

[1] Chhalliyil P, Ilves H, Kazakov S A, Howard S J, Johnston B H and J Fagan. 2020. A Real-Time Quantitative PCR Method Specific for Detection and Quantification of the First Commercialized Genome-Edited Plant. Foods. 9:1245

[2] Politico EU, 7 September 2020. EU countries may have a way to find gene-edited crops in imports

[3] Euroseeds, 8 September 2020, Much ado about nothing, really!

1 SU Canola

SU Canola is the commercial name for rapeseed lines engineered by US company Cibus to tolerate spraying with sulfonylurea and imidazolinone herbicides.

Commercial SU Canola varieties are derived from two GM events - 5715 and 5720:

- **Event 5715** is a cross between Cibus rapeseed line BnALS-57 and BASF Clearfield rapeseed line SP Cougar CL, according to the EUGinius database.[4]

  BnALS-57 was obtained using Cibus' proprietary version of oligonucleotide-directed mutagenesis (ODM) called "Rapid Trait Development System (RTDS)", whereas SP Cougar CL was created through "conventional breeding techniques".

  Event 5715 contains a single nucleotide mutation both in the AHAS1C gene and in the AHAS3A gene. The point mutation consists of a G to T change that confers tolerance to SU herbicides. BnALS-57 is the donor of the mutation in the AHAS1C gene, and SP Cougar CL is the donor of the same mutation in the AHAS3A gene.

- **Event 5720** is a re-transformation or re-mutation of event 5715, according to the Canadian Food Inspection Agency. Under Canadian Federal Law[5], a "retransformation" is a transformation of a plant with the identical construct(s) as a previously authorized plant of the same species, where as a "re-mutation" means the same mutation in a plant as in a previously authorized plant of the same species.

Currently, three SU Canola lines are available on the US market (32K, 40K and 68K) and two in Canada (68K and 79K). Canadian regulatory records document that 68K is derived from event 5715 while 79K is derived from event 5720. While no similar documentation is available on 32K and 40K, it is reasonable to assume that these cultivars are also derived from events 5715 or 5720.

2 GMO status depends on the techniques used

Under the EU GMO Directive, it is sufficient if a genetic modification "occurs through" the use of a GM technique (Art. 2(2)(a) of Directive 2001/18/EC).[6] That is, if a regulated GM technique has been applied in a development process and has altered the organism's genetic material in a way that does not occur naturally, the resulting organism is a GMO. The Directive neither requires that the development process is understood in detail nor that the mechanisms, interdependencies and causalities are clear and can be proven.

Furthermore, the definition of GMO in Art. 2 (2) of the Directive does not require that the intended modification of the genetic material is directly attributable to a specific genetic

[4] European GMO Initiative for a Unified Database System (EUGinius) [No Date] GMO 5715, SU Canola

[5] Directive 94-08 (Dir 94-08) Assessment Criteria for Determining Environmental Safety of Plants With Novel Traits. Appendix 1: Definitions.

[6] French: „se fait par", German: "kommt es durch".

engineering process. According to the ECJ, it follows from the general scheme of the Directive, that the list of techniques in part 1 of Annex I A to the Directive, which are considered to lead to genetic modification, is not exhaustive, while the lists of techniques in part 2 of Annex I A and the list of techniques of genetic modification yielding organisms to be excluded from the Directive in Annex I B to the Directive are exhaustive.[7]

Therefore, it is sufficient that the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination. The manner of alteration (i.e. the technique) only matters if it is exclusively one of the techniques listed in part 2 of Annex I A or in Annex I B. ODM does not qualify for such an exemption, according to the ECJ ruling.

3 Evidence that a regulated GM technique was used to create SU Canola

There is substantial documentation on the public record confirming that ODM, a gene editing technique, was used in the development of SU Canola. That documentation includes:

- Scientific literature authored by the developer
- Regulatory statements posted by Health Canada and, until July 2020, Canadian Food Inspection Agency (CFIA)
- Patent applications submitted by Cibus
- Institutional databases such as EUginius and the CBD Biosafety Clearinghouse

3.1 Scientific literature authored by the developer

Cibus states that SU Canola is a product of gene editing in three articles it authored over the period 2014-2017:

> **2014** - *"One method of practicing RTDS involves regenerating protoplasts to whole plants. By applying the process described in Figure 2 [RTDS process to develop herbicide tolerance in canola], mutations were obtained in canola AHAS homologs, one of which was key to developing Cibus' herbicide-tolerant SU Canola™ product, which is currently in the launch phase in the United States."*[8]
>
> **2015** - *"Concerted efforts at Cibus for more than a decade have advanced this technique in Arabidopsis as a model system and in OSR to develop the first commercial*

[7] ECJ, judgment of 25.07.2018, C-528/16, Confédération paysanne et al.; ECLI:EU:C:2018:583, paragraph 31 et seq.

[8] Gocal G W. 2014. *Non-Transgenic Trait Development in Crop Plants Using Oligo-Directed Mutagenesis: Cibus' Rapid Trait Development System*. In: New DNA-Editing Approaches: Methods, Applications and Policy for Agriculture. NABC Report No 26

product using this technique. To our knowledge this is the first report of gene editing in OSR."[9]

2017 - *"Cibus' SU Canola™, which the US Department of Agriculture (USDA) views as non-genetically modified (non-GM), is Cibus' first commercial product arising from plant genome editing and had its test launch in 2014."*[10]

3.2 Regulatory records

In 2014, Canadian federal agencies Health Canada and the Canadian Food Inspection Agency (CFIA) approved Cibus event 5715 and varieties derived from it for environmental release and human consumption.

In its Novel Food decision, **Health Canada** provides a description of the method used to develop event 5715, which clearly includes the application of ODM:

> *As donor of the single nucleotide mutation in the BnAHAS1C gene,* ***Cibus canola line BnALS-57 was selected as a mutant variant of the wild-type parental line BN2. Mutants of BN2 were generated using the Rapid Trait Development System (RTDS); an oligonucleotide-directed mutagenesis method.*** *A critical step of the RTDS protocol is the application of tissue culture techniques to generate plant cells more receptive to mutagenesis.* ***BN2 protoplasts were subjected to the RTDS*** *then cultured on media containing imazethapyr (an imidazolinone herbicide).* [11] [Emphasis added]

Until July 2020, the **Canadian Food Inspection Agency**'s determination approving environmental release of SU Canola similarly documented the use of ODM in the development of event 5715:

> *Cibus Canada Inc. utilized an oligonucleotide-directed mutagenesis approach known as the Rapid Trait Development System™ (RTDS™), which included the application of tissue culture techniques that generated plant cells more receptive to mutagenesis.* ***Following treatment of protoplasts of the BN2 parental canola line with the RTDS, a canola event known as BnALS-57 was isolated.*** *Sequencing confirmed that the BnAHAS1 gene of BnALS-57 contains a single nucleotide mutation, which confers tolerance to AHAS-inhibiting herbicides such as the sulfonylureas and imidazolinones.*[12] [Emphasis added]

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[9] Gocal G F W, Schöpke C and P R Beetham. 2015. Oligo-Mediated Targeted Gene Editing. In: Zhang F, Puchta, H and J G Thomson (ed). Advances in New Technology for Targeted Modification of Plant Genomes

[10] Songstad D D, Petolino J F, Voytas D F & N A Reichert. 2017. Genome Editing of Plants. In: Critical Reviews in Plant Sciences, 36:1, 1-23, DOI: 10.1080/07352689.2017.1281663

[11] Health Canada. 2016. Novel Food Information - Cibus Canola Event 5715 (Imidazolinone and Sulfonylurea Herbicide Tolerant)

[12] Canadian Food Inspection Agency. 2017. DD 2013-100: Determination of the Safety of Cibus Canada Inc.'s Canola (Brassica napus L.) Event 5715.

Both decisions, by Health Canada and the Canadian Food Inspection Agency, also reported the company's hypothesis that the mutation in BnALS-57 may not be due to the (specific) oligonucleotide used but due to "a spontaneous somaclonal variation that occurred during the tissue culture process". This hypothesis is addressed in Section 4 below.

In July 2020, the Canadian Food Inspection Agency issued a revised decision that removed reference to the application of ODM.[13] No scientific evidence has been provided in support of the revision and the CFIA has not provided any explanation for the revision.

3.3 'SU Canola' patent application

The key US patent application for SU Canola clearly documents that BnALS-57 was isolated from a culture to which an ODM process had been applied.[14]

The application describes how mutagenic oligonucleotides were introduced into canola protoplasts and variants with tolerance to an imidazolinone herbicide were subsequently selected and further characterized by the mutations that conferred herbicide resistance. (Example 1, paras 0084-0103). A specific isolate, identified as BnALS1621N-57 (also referred to as BnALS-57), was shown to carry the W547L mutation in the AHAS1C gene, the genetic alteration that is characteristic of SU Canola (paras 0096-0097).

3.4 Official GMO registers

Published in May 2016, the listing for SU Canola in the **Biosafety Clearinghouse (BCH)** of the **UN Convention on Biological Diversity** states that the technique used to achieve the modification was ODM:

> "*Cibus Canada Inc. utilized an oligonucleotide-directed mutagenesis approach known as the Rapid Trait Development System™ (RTDS™), which included the application of tissue culture techniques that generated plant cells more receptive to mutagenesis.*" [15]

The entry in the **European GMO database, EUginius,** similarly lists BnALS-57 as obtained using ODM[16]:

> "***BnALS-57 was selected as a mutant variant of the wild-type parental line BN2. Mutants of BN2 were generated using an oligonucleotide-directed mutagenesis (ODM) approach known as the Rapid Trait Development System™*** *(RTDS™). No*

[13] Canadian Food Inspection Agency. 2020. DD 2013-100: Determination of the Safety of Cibus Canada Inc.'s Canola (Brassica napus L.) Event 5715. Last updated July 10.
[14] US 2020/0123563 Mutated Acetohydroxyacid Synthase Genes in Brassica. Also see its antecedent, US 2012/0178628A1, Mutated Acetohydroxyacid Synthase Genes in Brassica.
[15] Biosafety Clearinghouse. 2016. Modified Herbicide Tolerant SU Canola. LMO Information. Record ID 110268. First entered May 5 2016. Last updated February 6 2020. The CBD and EUginius listings are based on the Canadian regulatory records.
[16] EUGinius [No Date] GMO 5715, SU Canola
The database is run by the German Federal Office of Consumer Protection and Food Safety (BVL) and Dutch Wageningen Food Safety Research of Wageningen University and Research (Wageningen UR).

changes were observed in the promoter regions or locations that would affect endogenous gene expression. This was confirmed by sequencing a 2500-base pair (bp) region upstream of the AHAS1 *gene which was determined to be identical with GenBank accession Z11524."* [Emphasis added]

4 Off-target effects of ODM are likely responsible for SU Canola

Cibus contends that the desired mutation conferring herbicide tolerance at a specific location in the SU Canola genome is not related to the application of the ODM technique but occurred spontaneously during the cell culture process.

However, careful examination of the company's own data on SU Canola indicates that the mutation conferring herbicide tolerance in Bn-ALS-57 is likely to be an off-target effect directly caused by application of ODM.

Although the documentation in the public domain remains incomplete, certain conclusions can be drawn with confidence:

- Experimental line BnALS-57 is the outcome of an ODM process that resulted in changes at a different site in the genome than the company intended. The oligonucleotide used in this process was designed to bring about the S653N mutation, not the G to T change characteristic of SU Canola (W547L mutation).
- It is well established that ODM gives rise to off-target effects and that the mechanisms operating in ODM are very poorly understood and lead to variable outcomes.[17,18]
- The high levels of oligonucleotides introduced into cells as part of the ODM process trigger DNA damage signalling and activate DNA repair, which can be mutagenic.[19, 20]
- It is apparent from Cibus' patent application that the specific ODM process used by the company significantly increases the rate of random mutagenesis. The application shows accelerated mutagenesis at the site where the G to T change happened. This site is less than 100 base pairs away from the site where the oligonucleotide was meant to bind.[21]

[17] Dong, C.; Sharp, P. Oligonucleotide-Directed Gene Repair: Promises and Limitations for Plant Gene Modification. Transgenic Plant Journal 2007, 1(1), 10–16

[18] Eckerstorfer, M.; Miklau, M.; Gaugitsch, H.; Oesterreich; Umweltbundesamt *New plant breeding techniques and risks associated with their application*; 2014; ISBN 978-3-99004-282-3.

[19] Ferrara, L.; Kmiec, E.B. Targeted gene repair activates Chk1 and Chk2 and stalls replication in corrected cells. DNA Repair 2006, 5, 422–431, doi:10.1016/j.dnarep.2005.11.009

[20] Igoucheva, O.; Alexeev, V.; Yoon, K. Differential cellular responses to exogenous DNA in mammalian cells and its effect on oligonucleotide-directed gene modification. Gene Ther 2006, 13, 266–275, doi:10.1038/sj.gt.3302643

[21] US 2020/0123563 Mutated Acetohydroxyacid Synthase Genes in Brassica

- On the basis of the above observations, it is reasonable to conclude that BnALS-57 is the result of off-target effects of ODM, and that Cibus' claims regarding the genesis of BnALS-57 are not supported by the evidence.

5 Conclusion: SU Canola is a GMO

An organism comes under EU GMO law if a regulated GM technique was used to develop it, and the development process has altered the organism's genetic material in a way that does not occur naturally.

It is well documented that (ODM) was used to develop SU Canola and this is a regulated GM technique.

The Commission and EU member states should now be implementing measures to ensure this unauthorised product does not enter the EU's food supply. This includes initiating a detection regime to screen for SU Canola, relying on the best method available.

Prompt action is required as SU Canola is the first commercial gene-edited crop and so a test case for responding under EU law. To accept Cibus' narrative would send a signal to GM developers that they can evade regulation by creative interpretation of the law, and so undermine regulatory and legal authority.

Contacts:

Florian Faber, Managing Director, **ARGE Gentechnik-frei,** +43 664 3819502, f.faber@gentechnikfrei.at

Dirk Zimmermann, Campaigner for sustainable agriculture, **Greenpeace**, +49 40 30618-339, dirk.zimmermann@greenpeace.org

Alexander Hissting, Managing Director, **Verband Lebensmittel ohne Gentechnik e.V.** (VLOG), +49 30 2359 945 10, a.hissting@ohnegentechnik.org