Risk assessment of new sequencing information for genetically modified sugar beet H7-1

European Food Safety Authority (EFSA), Paolo Lenzi, Nikoletta Papadopoulou and Tommaso Raffaello

Abstract

The EFSA Panel on genetically modified organisms (GMO Panel) has previously assessed genetically modified (GM) sugar beet H7-1. This sugar beet was found to be as safe and nutritious as its conventional counterpart and commercial sugar beet varieties with respect to potential effects on human and animal health and the environment in the context of its intended uses. On 19 February 2021, European Commission requested EFSA to analyse new nucleic acid sequencing data and updated bioinformatics data for GM sugar beet H7-1 and to indicate whether the previous conclusions of the GMO Panel on safety of GM sugar beet H7-1 remain valid. The new sequencing data indicated seven nucleotide differences as compared to the sequence originally provided in applications EFSA-GMO-UK-2004-08 and EFSA-GMO-RX-006: five nucleotides in the 5’ genomic flanking region and two nucleotides in the T-DNA region of the insert. Two mismatches between the originally submitted H7-1 event sequence and the plasmid sequence were confirmed by the newly obtained H7-1 sequence. Based on the analysis of the provided data, EFSA considers that the newly reported sequence differences are most likely attributed to sequencing errors in the originally reported sugar beet H7-1 event sequence. The new sequencing data and the bioinformatic analyses performed on the new sequence did not give rise to safety issues. Therefore, EFSA concludes that the original risk assessment of sugar beet H7-1 remains valid.

© 2022 Wiley-VCH Verlag GmbH & Co. KgaA on behalf of the European Food Safety Authority.

Keywords: GMO, sugar beet, H7-1, nucleotide sequence, Regulation (EC) No 1829/2003

Requestor: European Commission

Question number: EFSA-Q-2021-00135

Correspondence: nif@efsa.europa.eu
Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

Acknowledgments: EFSA wishes to thank the following for the support provided to this scientific output: Nils Rostoks and Antonio Fernandez Dumont.

Suggested citation: EFSA (European Food Safety Authority), Lenzi P, Papadopoulou N and Raffaello T, 2022. Statement on risk assessment of new sequencing information for genetically modified sugar beet H7-1. EFSA Journal 2022;20(6):7354, 6 pp. https://doi.org/10.2903/j.efsa.2022.7354

ISSN: 1831-4732

© 2022 Wiley-VCH Verlag GmbH & Co. KgaA on behalf of the European Food Safety Authority.

This is an open access article under the terms of the Creative Commons Attribution-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited and no modifications or adaptations are made.

The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.
# Table of contents

Abstract

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

2. Data and methodologies

2.1. Data

2.2. Methodologies

2.2.1. Sequence information previously submitted to EFSA for sugar beet H7-1

2.2.2. New information for GM sugar beet event H7-1 submitted as part of the current mandate

3. Assessment

4. Conclusions

5. Documentation as provided to EFSA

References

Abbreviations
1. **Introduction**

Genetically modified (GM) sugar beet H7-1 was obtained by *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*)-mediated transformation with plasmid PV-BVG T08. The T-DNA region of the plasmid contains the *cp4 epsps* expression cassettes, conferring glyphosate tolerance. The *cp4 epsps* expression cassette consists of the following elements: the 35S promoter from a modified figwort mosaic virus (P-FMV), a chloroplast targeting sequence from *Arabidopsis thaliana* (*ctp2*), the EPSPS coding sequence from *Agrobacterium sp.* strain CP4 (*cp4 epsps*) and the E9 3’ polyadenylation signal from the pea *rbcS* E9 gene (*Pisum sativum*).

The GMO Panel has previously assessed GM sugar beet H7-1 in the frame of applications EFSA-GMO-UK-2004-08 (EFSA, 2006) and EFSA-GMO-RX-006 (EFSA GMO Panel, 2017). This European Food Safety Authority (EFSA) statement assesses the additional sequencing information received for the GM sugar beet event H7-1.

1.1. **Background and Terms of Reference as provided by the requestor**

On 22 February 2019, the European Commission (EC) received from Bayer and KWS new sequencing information related to sugar beet event H7-1, on the basis of Articles 9 and 21 of Regulation (EC) 1829/2003. On 19 February 2021, the EC requested EFSA to evaluate the data and analyses provided by Bayer and KWS and indicate whether, on the basis of these elements, the conclusions of the adopted opinion for GM sugar beet H7-1 remain valid. Subsequently, EFSA has evaluated the data and methodology provided for GM sugar beet H7-1 and considered these elements in the context of previous conclusions.

2. **Data and methodologies**

2.1. **Data**

In delivering this statement, EFSA took into account information provided by the applicant and relevant scientific publications.

2.2. **Methodologies**

The applicant followed the relevant parts of the GMO Panel guideline for the risk assessment of GM plants (EFSA GMO Panel, 2011) and Regulation (EU) No 503/2013 to investigate the insert sequence and to perform the bioinformatics analyses. In delivering this statement, EFSA took into account the appropriate principles described in the GMO Panel guidelines for the risk assessment of GM plants (EFSA GMO Panel, 2011), Regulation (EU) No 503/2013 and Directive 2001/18/EC.

2.2.1. **Sequence information previously submitted to EFSA for sugar beet H7-1**

The applicant had previously submitted information on the sequence of GM sugar beet event H7-1, as part of applications EFSA-GMO-UK-2004-08 (EFSA, 2006) and EFSA-GMO-RX-006 (EFSA GMO Panel, 2017). Sugar beet H7-1 contains a single insert. The 3354-bp insert contains one *cp4 epsps* expression cassette, consisting of the P-FMV promoter from the figwort mosaic virus, the *ctp2* chloroplast targeting sequence from *Arabidopsis thaliana*, the *cp4 epsps* synthetic gene based on the sequence from *Agrobacterium sp.* strain CP4 and the E9 3’ polyadenylation region from the pea *rbcS* E9 gene (*Pisum sativum*). In addition, 789 and 806 bp of the 5’ and 3’ flanking regions, respectively, were sequenced in 2011 and assessed in EFSA-GMO-RX-006 (EFSA GMO Panel, 2017).

2.2.2. **New information for GM sugar beet event H7-1 submitted as part of the current mandate**

The applicant re-sequenced the single GM sugar beet event H7-1 and compared this sequence with the original sugar beet event sequence reported in application EFSA-GMO-UK-2004-08 (EFSA, 2006) and

---

1. Commission Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. OJ L157, 8.6.2013, p. 1 – 48.

2. Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 17.4.2001, p. 1 – 39.
EFSA-GMO-RX-006 (EFSA GMO Panel, 2017). On 25 January 2021, EURL-GMFF verified and concluded that the sequence methodology of the GM event H7-1 complies with the requirements of the updated Joint Research Centre (JRC) Guideline for the submission of DNA sequences\(^3\) within the framework of Regulation (EC) No 1829/2003\(^4\). This comparison revealed a number of sequence differences listed in Table 1: Five nucleotide differences in the 5' flanking region between positions –759 and –750; two nucleotide differences within the T-DNA at positions 1,093 and 3,247. The change at position 1,093 is a synonymous mutation occurring within the cp4 coding region and does not change the amino acidic sequence of the translated protein; the change reported at position 3247 is an insertion and it occurs in an intragenic region of the T-DNA. These two positions within the T-DNA of the newly submitted sequence do not differ from the sequence of the transformation plasmid reported in EFSA-GMO-UK-2004-08 (EFSA, 2006).

Table 1: Identified differences in the sequence of the inserts and flanking regions in sugar beet event H7-1.

| Identified difference | Position* | Sequence in plasmid PV-BVGT08 | Reported in application EFSA-GMO-UK-2004-08 | Reported in the current mandate |
|-----------------------|-----------|--------------------------------|--------------------------------------------|-------------------------------|
| 5' Flanking region    | –759      | n.a.                           | GGATCCCAAGA                                | CGGGCCCAAGG                   |
|                       | –757      | n.a.                           | GGATCCCAAGA                                | CGGGCCCAAGG                   |
|                       | –756      | n.a.                           | GGATCCCAAGA                                | CGGGCCCAAGG                   |
|                       | –753      | n.a.                           | GGATCCCAAGA                                | CGGGCCCAAGG                   |
|                       | –750      | n.a.                           | GGATCCCAAGA                                | CGGGCCCAAGG                   |
| T-DNA insert          | 1,093     | GAAACTCGTAT                    | GAAACTCGTAT                                | GAAACTCGTAT                   |
|                       | 3,247     | ATTAGAAAAAAT                  | ATTAGAAAAAAT                               | ATTAGAAAAAAT                  |

*: Positions are defined as reported in the original submission EFSA-GMO-UK-2004-08 (EFSA, 2006).

Two mismatches with the plasmid were reported in the original sequence at position 622 and 2,457, and were confirmed in the newly submitted sequence.

Genomic DNA used for the sequencing of the original GM sugar beet H7-1 event was isolated from plant material deriving from a homozygous T2 generation obtained by self-pollination from the homozygous T1. Genomic DNA used for the new sequencing experiment was isolated from a hybrid derived from the same homozygous T1 generation. This was obtained by several crosses and backcrosses with the elite line.

Although random mutations may have occurred between the two lines, it is unlikely that five nucleotide changes could have occurred in such a short region (–759 to –750), with an absence of high-density polymorphism in other regions of the insert. Moreover, the reported nucleotide differences are present at the beginning of the sequence, where sequencing errors are most likely to occur.

Regarding the other two changes, at position 1,093 and 3,247, the nucleotides in the newly submitted sequence do not differ from the transformation plasmid; the data indicate that these differences are technical errors that occurred during amplification.

The applicant provided a complete bioinformatics data set using the updated H7-1 event sequence including an analysis of the insert and flanking sequences, an analysis of the potential similarity to allergens and toxins of the newly expressed protein and of all possible open reading frames (ORFs) within the insert and spanning the junction sites, and an analysis of possible horizontal gene transfer (HGT).

3. Assessment

The applicant resequenced the sugar beet H7-1 event and compared this sequence with the originally submitted sugar beet H7-1 event sequence, which revealed seven nucleotide differences. The new sequencing information was evaluated by the JRC and was found to be compliant with the JRC guideline.

Based on the analysis of the provided information, it can be concluded that the reported differences can most likely be attributed to sequencing errors in the originally reported sugar beet H7-1 event sequence, rather than spontaneous mutations.

---

3 https://gmo-crl.jrc.ec.europa.eu/doc/Guideline-Sequencing-Feb-2016-mod-April-2017.pdf
4 Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed.
Bioinformatic analyses for the potential disruption of any known sugar beet genes were performed by the applicant. The data indicate that the 5’ insertion site is located in a region with similarity to a gene annotated as ‘DETOXIFICATION 42’, likely to be interrupted. This is in line with what previously assessed in application EFSA-GMO-RX-006. The agronomic, phenotypic and compositional analyses of the GM plants did not indicate biologically relevant differences that can be attributed to the function of a specific single gene that would raise any safety concerns.

Bioinformatic analyses performed on all putative ORFs defined from stop-to-stop codon generated by the new sequence information with regard to potential similarity with allergens or toxins were performed according to EFSA guidelines (EFSA GMO Panel, 2011). Results indicate that none of the newly generated ORFs show significant similarity with known toxins and allergens. Sequence analysis using the updated H7-1 event sequence did not identify any similarity with microbial sequences. Therefore, the updated H7-1 sequence does not affect the likelihood of HGT.

4. Conclusions

Resequencing of the sugar beet H7-1 event by the applicant, and comparison with the originally submitted sequence, revealed seven nucleotide differences. Based on the analysis of the provided and additionally requested data, EFSA considers that the reported sequence differences are most likely attributed to sequencing errors in the originally reported sugar beet H7-1 event sequence. In addition, two mismatches between the original plant sequence and the transformation plasmid were already reported in the original application and were confirmed by the newly submitted sequence.

Studies other than bioinformatics are not affected by the new sequence information. The bioinformatic analyses using the newly determined H7-1 sequence did not give rise to safety issues. Therefore, EFSA concludes that the original risk assessment of the GM sugar beet H7-1 remains valid.

5. Documentation as provided to EFSA

1) Mandate from the European Commission received on 19 February 2021 concerning a request to analyse new sequencing information for sugar beet H7-1, provided by Bayer and KWS.
2) Mandate accepted by EFSA on 19 March 2021.
3) Request for supplementary information (1) to the applicant, sent on 26 March 2021.
4) Request by applicant for a deadline extension to the response (1) to 30 October, received on 20 April 2021.
5) Deadline extension acceptance by EFSA, 30 April 2021.
6) Receipt of supplementary information (1) from the applicant on 27 October 2021.
7) Request for supplementary information (2) from the applicant, sent on 21 December 2021.
8) Receipt of supplementary information (2) from the applicant on 21 February 2022.
9) Request to EC to extend the deadline of the mandate, to 9 June 2022, sent on 11 March 2022.
10) Acceptance of the deadline extension requested to EC, 25 March 2022.

References

EFSA (European Food Safety Authority), 2006. Opinion of the Scientific Panel on Genetically Modified Organisms on an application (reference EFSA-GMO-UK-2004-08) for the placing on the market of products produced from glyphosate-tolerant genetically modified sugar beet H7–1, for food and feed uses, under Regulation (EC) No1829/2003 from KWS SAAT and Monsanto. EFSA Journal 2006;4(12):431, 18 pp. https://doi.org/10.2903/j.efsa.2006.431

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011. Scientific Opinion on Guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal 2011;9(5):2150, 37 pp. https://doi.org/10.2903/j.efsa.2011.2150

EFSA GMO Panel (EFSA Panel on genetically modified organisms), 2017. Scientific Opinion on the assessment of genetically modified sugar beet H7–1 for renewal of authorisation under Regulation (EC) No 1829/2003 (application EFSA-GMO-RX-006). EFSA Journal 2017;15(11):5065, 9 pp. https://doi.org/10.2903/j.efsa.2017.5065

Abbreviations

GM genetically modified
GMO genetically modified organism
HGT horizontal gene transfer
JRC Joint Research Centre
ORF open reading frames