EFSA STATEMENT

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Risk assessment of new sequencing information for genetically modified soybean BPS-CV127-9

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Abstract

The GMO Panel has previously assessed genetically modified (GM) soybean BPS-CV127-9. This soybean was found to be as safe and nutritious as its conventional counterpart and commercial soybean varieties with respect to potential effects on human and animal health and the environment in the context of its intended uses. On 16 February 2018, European Commission requested EFSA to analyse new nucleic acid sequencing data and updated bioinformatics data for GM soybean BPS-CV127-9 and to indicate whether the previous conclusions of the GMO Panel on safety of GM soybean BPS-CV127-9 remain valid. The new sequencing data indicated a two nucleotide difference in the unannotated *Arabidopsis* genomic DNA sequence downstream of the 3′ untranslated region of the *ahasl* (also referred to as *csr1-2*) gene as compared to the sequencing data originally provided. One of these nucleotide differences reported in the new nucleic acid sequencing data from GM soybean event BPS-CV127-9 was shown to be already present in the original plant material used for the risk assessment. However, for the other nucleotide difference reported, no evidence could be provided to differentiate between a sequencing error and point-mutation. With the exception of bioinformatics analyses, the studies performed for the risk assessment of the single event soybean BPS-CV127-9 remain valid. The new sequencing data and the bioinformatics analyses performed on the new sequence did not give rise to safety issues. Therefore, EFSA concludes that the original risk assessment of soybean BPS-CV127-9 remains valid.

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Keywords: GMO, soybean, BPS-CV127-9, nucleotide sequence, regulation (EC) no 1829/2003

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1. Introduction

Genetically modified (GM) soybean BPS-CV127-9 was obtained via particle bombardment of a purified, linear DNA fragment derived from plasmid pAC321 and contains the *ahasl* (referred to as *csr1-2* hereafter) gene cassette, encoding for the aceto-hydroxy acid synthase (AHAS) large subunit. The AHAS enzyme catalyses the first step in the biosynthesis of branched-chain amino acids, and the enzyme encoded by the *csr1-2* mutant allele retains the normal catalytic activity while preventing the binding of imidazolinone herbicides.

The GMO Panel has previously assessed GM soybean BPS-CV127-9 as part of application EFSA-GMO-NL-2009-64 (EFSA GMO Panel, 2014). This EFSA statement assesses the additional sequencing information received for the GM soybean event BPS-CV127-9.

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

On 27 October 2017, the European Commission received from BASF new sequencing information related to soybean event BPS-CV127-9, on the basis of Articles 9 and 21 of Regulation (EC) 1829/2003. On 16 February 2018, the European Commission requested the European Food Safety Authority (EFSA) to evaluate the data and analyses provided by BASF and indicate whether, on the basis of these elements, the conclusions of the adopted opinion for GM soybean BPS-CV127-9 remain valid. Subsequently, EFSA has evaluated the data and methodology provided for GM soybean BPS-CV127-9 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 guidelines for the risk assessment of GM plants (EFSA GMO Panel, 2011) 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 genetically modified (GM) plants (EFSA GMO Panel, 2011) and Regulation (EU) No 503/2013[1].

2.3. Sequence information previously submitted to EFSA for GM soybean event BPS-CV127-9

The applicant had previously submitted information on the sequence of GM soybean event BPS-CV127-9, as part of application EFSA-GMO-NL-2009-64 (EFSA GMO Panel, 2014). The 4,758 bp insert contained a rearranged fragment of *Arabidopsis* genomic DNA consisting of 17 bp of unannotated *Arabidopsis* genomic DNA, the *Arabidopsis* gene *AtSEC61*γ (locus At3g48570) that encodes the transport protein Sec61 with 62 bp of its 5′ leader sequence upstream of the start codon, a mutant allele (S653N) of the *Arabidopsis* gene *csr1-2*, with its 5′ and 3′ flanking regions and a 376 bp fragment corresponding to a partial duplication of the *csr1-2* gene. Sequence analysis indicated that the *csr1-2* coding sequence in the GM soybean event BPS-CV127-9 differs from the *Arabidopsis* donor sequence by a single nucleotide that results in one amino acid replacement (arginine to lysine at position 272). Two additional differences compared to the vector pAC321 sequence are located downstream of the 3′ untranslated region of the *csr1-2* gene.

[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.

2.3.1. New information for GM soybean event BPS-CV127-9 submitted as part of the current mandate

The applicant has recently re-sequenced the single GM soybean event BPS-CV127-9 and compared this sequence with the original soybean event sequence reported in 2009.[2] This revealed a two nucleotide difference in the unannotated *Arabidopsis* genomic DNA downstream of the 3′ untranslated region of the *csr1-2* gene as compared to the sequencing data originally provided (see Table 1).

Table 1: Identified differences in the sequence of the inserts and flanking regions in soybean event BPS-CV127-9

Identified difference	Position new BPS-CV127-9 sequence*	Reported in 2009	Reported in 2017
Arabidopsis gDNA, unannotated	5255	ATCC**A**AAAAAAAAAAAAAAAAAT	ATCC_AAAAAAAAAAAAAAAAT
Arabidopsis gDNA, unannotated	5531	TA**G**A	TA**A**A

*: Positions are relative to each insertion region.

Genomic DNA from pooled seeds of breeding line BRS397CV, produced by conventional breeding from the F8 generation of CV127 line 603, was used to generate the new sequence of event BPS-CV127-9. The original 2009 sequence was generated from genomic DNA from leaf tissue from CV127 line 603 F7 plants.[2] As the new sequence information was not generated from the same material used to sequence the original material, the applicant provided evidence that the reported G to A nucleotide difference between the original and new sequence was already present in the original sequence. However, the applicant could not provide evidence that the 17 adenines found in the newly generated BPS-CV127-9 sequence were already present in the originally provided BPS-CV127-9 event sequence, where 18 adenines were originally reported. Therefore, it could not be concluded that the reported nucleotide difference was already present in the original sequence.

For the reported differences, the applicant evaluated the impact on the original bioinformatics analyses.[3] For GM soybean event BPS-CV127-9, containing the two reported differences, the applicant carried out bioinformatics analyses using the updated nucleotide sequence in order to investigate (1) if any open reading frame (ORF) present within the insert shows similarity to known allergens or toxins, and (2) if the insert contains sequences that would facilitate horizontal gene transfer (HGT) to microorganisms. The applicant indicated that the two reported nucleotide differences did not affect the ORFs spanning the junctions between the insert and genomic DNA and were therefore not analysed.

3. Assessment

The provided data indicated that one of the sequence differences in GM soybean event BPS-CV127-9 was already present in the original material used in application EFSA-GMO-NL-2009-64 (EFSA GMO Panel, 2014). For the other nucleotide difference, the applicant could not provide evidence that the 17 adenines found in the newly generated BPS-CV127-9 sequence were already present in the originally provided BPS-CV127-9 event sequence, where 18 adenines were originally reported. The applicant indicated that sequencing of homopolymeric adenine stretches is technically challenging and could explain the discrepancy between the new and old sequence. The likelihood of a misread in a homopolymeric adenine stretch is likely higher than a spontaneous mutation in this region.[4] However, because BPS-CV127-9 material from the original sequencing is not available anymore, no definitive conclusion could be reached to whether the 17 adenines were already present in the original sequence or whether the 17 adenines are the result of a spontaneous deletion.

Bioinformatics analyses performed with the updated sequence of GM soybean event BPS-CV127-9 with regard to potential similarity with allergens or toxins, as well as the implications of these differences on the potential for HGT, were considered relevant for the current assessment. The bioinformatics searches for similarity to allergens were performed according to EFSA guidelines (EFSA

[2] BASF Reg.Doc. No 2017/7015991 (confidential information).
[3] BASF Reg.Doc. No 2017/7015991 (confidential information) and additional information 10-4-2018.
[4] Additional information 14-5-2018.

GMO Panel, 2010, 2011). Results indicate that none of the ORFs, newly generated by the reported nucleotide differences, show similarity with known allergens or toxins. Sequence analysis using the updated BPS-CV127-9 event sequence did not identify any similarity with microbial sequences. Therefore, the updated BPS-CV127-9 sequence does not affect the likelihood of HGT.

The other studies performed for the risk assessment of GM soybean event BPS-CV127-9 are not affected by the new sequencing information.

4. Conclusions

Based on the analysis of the provided data, it can be concluded that one of the nucleotide differences reported for GM soybean event BPS-CV127-9 was present in the original material used for the risk assessment of the single event GM soybean BPS-CV127-9. Based on the provided information, it could not definitely be concluded that the 17 adenines found in the newly generated BPS-CV127-9 sequence were already present in the originally provided BPS-CV127-9 event sequence, where 18 adenines were originally reported. The bioinformatics analyses with the new sequence did not give rise to safety issues. Studies other than bioinformatics are not affected by this new sequence information. EFSA concludes that the original risk assessment of the single GM soybean BPS-CV127-9 remains valid.

Documentation provided to EFSA

1) Letter from the European Commission received on 16 February 2018 concerning a request to analyse new sequencing information for soybean BPS-CV127-9.
2) Acknowledgement letter dated 6 March 2018 from EFSA to the European Commission.
3) Letter from EFSA to applicant dated 28 March 2018 requesting additional information.
4) Letter from applicant to EFSA received on 10 April 2018 providing additional information.
5) Letter from EFSA to applicant dated 4 May 2018 requesting additional information.
6) Letter from applicant to EFSA received on 14 May 2018 providing additional information.
7) Letter from EFSA to applicant dated 7 June 2018 requesting additional information.
8) Letter from applicant to EFSA received on 14 June 2018 providing a timeline for responses.
9) Letter from applicant to EFSA received on 20 August 2018 providing additional information.

References

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010. Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 2010;8(7):1700, 168 pp. https://doi.org/10.2903/j.efsa.2010.1700

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), 2014. Scientific Opinion on application (EFSA-GMO-NL-2009-64) for the placing on the market of herbicide-tolerant genetically modified soybean BPS-CV127-9 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from BASF Plant Science. EFSA Journal 2014;12(1):3505, 30 pp. https://doi.org/10.2903/j.efsa.2014.3505

Abbreviations

AHAS	aceto-hydroxy acid synthase
bp	base pair
GM	genetically modified
GMO	genetically modified organism
HGT	horizontal gene transfer
ORF	open reading frame