EFSA STATEMENT

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Risk assessment of new sequencing information for genetically modified soybean A2704-12

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Abstract

The GMO Panel has previously assessed genetically modified (GM) soybean A2704-12. This soybean was found to be as safe and nutritious as its conventional counterpart with respect to potential effects on human and animal health and the environment in the context of its intended uses. On 5 June 2018, the European Commission requested EFSA to analyse new nucleic acid sequencing data and updated bioinformatics data for GM soybean A2704-12 and to indicate whether the previous conclusions of the GMO Panel on the risk assessment of GM soybean A2704-12 remain valid. The new sequencing data indicated seven nucleotide differences as compared to the sequence originally provided in application EFSA-GMO-NL-2005-18; six nucleotides in the 3′ genomic flanking region and one nucleotide in the soybean chloroplast DNA fragment located 5′ to the insert. Another nucleotide located in a polylinker region of the insert reported as ambiguous in the originally submitted sequence was resolved in the new sequence data. Based on the information provided on the locations affected by the reported nucleotide differences, no open reading frames (ORFs) spanning the junction site between the insert and the 3′ genomic flanking DNA are affected by the differences in the 3′ genomic flanking region and were therefore excluded from the assessment. However, one ORF spanning the junction between the 5′-chloroplast DNA fragment and the insert and six ORFs overlapping with the resolved nucleotide in the polylinker region were affected and therefore assessed. Based on the analysis of the provided data, EFSA considers that it is highly unlikely that the reported sequence differences (including the single nucleotide difference in the 5′-chloroplast DNA fragment) are due to spontaneous mutations and can therefore most likely be attributed to sequencing errors in the originally reported soybean A2704-12 event sequence. With the exception of bioinformatic analyses, the studies performed for the risk assessment of soybean A2704-12 are not affected. 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 soybean A2704-12 remains valid.

Keywords: GMO, soybean, A2704-12, nucleotide sequence, Regulation (EC) No 1829/2003

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1. Introduction

Genetically modified (GM) soybean A2704-12 was obtained via particle bombardment using a *Pvu*I digested pB2/35SAcK plasmid and contains the *pat* expression cassette. The *pat* expression cassette consists of the following elements: the P35S promoter from cauliflower mosaic virus, the synthetic *pat* gene derived from the *pat* gene of *Streptomyces viridochromogenes* and the T35S terminator from cauliflower mosaic virus. The *pat* gene encodes for the phosphinothricin acetyltransferase (PAT) protein conferring tolerance of soybean A2704-12 to glufosinate-ammonium based herbicides.

The GMO Panel has previously assessed GM soybean A2704-12 in the frame of application EFSA-GMO-NL-2005-18 (EFSA, 2007). This the European Food Safety Authority (EFSA) statement assesses the additional sequencing information received for the GM soybean event A2704-12.

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

On 24 August 2017, the European Commission (EC) received from Bayer new sequencing information related to soybean event A2704-12, on the basis of Articles 9 and 21 of Regulation (EC) 1829/2003. On 5 June 2018, the EC requested EFSA to evaluate the data and analyses provided by Bayer and indicate whether, on the basis of these elements, the conclusions of the adopted opinion for GM soybean A2704-12 remain valid. Subsequently, EFSA has evaluated the data and methodology provided for GM soybean A2704-12 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 genetically modified (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, 2010, 2011) and Regulation (EU) No 503/2013[1].

2.3. Sequence information previously submitted to EFSA for GM soybean event A2704-12

The applicant had previously submitted information on the sequence of GM soybean event A2704-12, as part of application EFSA-GMO-NL-2005-18 (EFSA, 2007). Soybean A2704-12 contains a single insert. The 6780-bp insert consists of two copies of the *pat* expression cassette and one copy each of the 5′ and 3′ parts of the *PvuI*-digested β-lactamase (*bla*) antibiotic resistance gene integrated between the two copies of the *pat* cassette. The 5′ part of the *bla* sequence was integrated in a reverse orientation with respect to the 3′ *bla* sequence part and hence the integration of these parts does not constitute a functional *bla* gene. In addition, ~4 kb and ~0.3 kb of the 5′ and 3′ flanking regions, respectively, were sequenced. The analysis of the 5′ flanking region indicated also the integration of a soybean chloroplast DNA fragment located 5′ to the insert (hereinafter referred to as 5′-chloroplast DNA fragment) (EFSA, 2007).

2.3.1. New information for GM soybean event A2704-12 submitted as part of the current mandate[2]

The applicant has recently sequenced the soybean event A2704-12 (insert and flanking regions) of an 'early-generation' soybean A2704-12 and a commercially available soybean A2704-12 variety (on the market since 2016). In addition, the applicant sequenced the insertion locus of the non-GM

[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] Study number: M-575181-03-1, M-593419-02-1, M-594978-01-1, M-617842-01-1 and additional information: 31/07/2018 and 24/09/18 (confidential information).

soybean A2704 variety (the same genetic background as the GM soybean A2704-12). The applicant also provided updated sequencing reports on the A2704-12 event sequence originally submitted in application EFSA-GMO-NL-2005-18.[3] The comparison of the newly determined 'early generation' soybean A2704-12 event sequence with the A2704-12 sequence originally submitted to EFSA (in the frame of application EFSA-GMO-NL-2005-18) revealed a number of sequence differences; six nucleotides in the 3′ soybean genomic flanking region and one nucleotide in the 5′-chloroplast DNA fragment (Table 1). The applicant clarified that the positions of the sequence differences in the 3′ soybean genomic flanking sequence fall outside the region spanning the junction between the genomic DNA and the insert giving rise to newly created open reading frames (ORFs). As regards the sequence difference in the 5′-chloroplast DNA fragment at position 3802, this leads to the generation of a STOP codon that initiates one of the newly created ORFs spanning the junction between the 5′-chloroplast DNA fragment and the insert resulting in an ORF that is nine amino acids shorter in the 'early generation' A2704-12 sequence than the corresponding ORF in the originally submitted event sequence.

Another nucleotide located in the polylinker region of the insert between T35S and the 3′ of the *bla* gene (position 6244) described as ambiguous (R) in the originally submitted A2704-12 event sequence is reported as a true base in the new sequencing data (see Table 1). The applicant clarified that six newly created ORFs overlap with position 6244 and are therefore affected by the new sequencing data.

The applicant performed further analyses and compared the early generation soybean A2704-12 event sequence with the event sequence of the commercially available soybean A2704-12 as well as with the non-GM insertion locus sequence corresponding to the flanking regions; results indicate that the respective sequences are 100% identical in both cases.

Table 1: Identified differences in the sequence of the insert and flanking regions in soybean event A2704-12

Identified difference	Position new A2704-12 sequence[(a)]	Reported in application EFSA-GMO-NL-2005-18	Reported in the current mandate[(b)]
Integrated soybean chloroplast DNA fragment	3802	CAC**C**GAT	CAC**T**GAT
Polylinker region in the inserted sequence	6244	AAG**R**ACA	AAG**A**ACA
3′ flanking sequence	10813	ATA**G**AGA	ATA**A**AGA
3′ flanking sequence	10866	AA**G**AA	AA**T**AA
3′ flanking sequence	10869-10872	AA**GGGG**AA	AA**TTTT**AA

(a): Positions are defined as reported for the early generation GM soybean A2704-12 sequence.
(b): The A2704-12 event sequences derived from the early generation GM soybean A2704-12 and from the soybean A2704-12 commercial variety are 100% identical.

In order to generate the event sequence of soybean A2704-12 defined by the applicant as 'early generation soybean A2704-12 sequence', genomic DNA from pooled seeds of soybean A2704-12 from the T4 generation (fourth generation after the original transformant) were used. The applicant clarified that it was not possible to precisely confirm the origin of the material used to generate the A2704-12 sequence originally submitted in application EFSA-GMO-NL-2005-18. However, based on the available information, the applicant could deduce that the material used was either of the same generation (T4) as the one used to generate the early generation soybean A2704-12 sequence or one generation earlier (T3, third generation after the original transformant). The sequence of the commercially available soybean A2704-12 variety was produced using seeds from GM plants obtained after multiple self-pollinated generations derived from the early generation soybean A2704-12. For the newly obtained insertion locus sequence, genomic DNA from pooled seeds of the non GM soybean A2704 variety (the same genetic background as the GM soybean A2704-12), were used.

The applicant provided a complete bioinformatics dataset using the updated A2704-12 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 ORFs within the insert and spanning the junction sites, and an analysis of possible horizontal gene transfer (HGT).

[3] Study number: M-216246-02-1 and M-216246-03-1 (confidential information).

3. Assessment

Based on the analysis of the information on the locations of the reported sequence differences, none of the newly created ORFs spanning the junction between the 3′ soybean genomic flanking DNA and the insert are affected by the sequence differences in the 3′ soybean genomic flanking region and were therefore excluded from the assessment.

On the other hand, one ORF spanning the junction between the 5′-chloroplast DNA fragment and the insert is affected by the single nucleotide sequence difference in the 5′-chlololplast DNA fragment and six ORFs are affected by the resolved base in the polylinker region of the insert. The bioinformatic analyses performed on these ORFs with regard to potential similarity with allergens or toxins as well as the implications of the newly determined event sequence on the potential for HGT were considered relevant for the current assessment. The results indicated that none of the possible ORFs (including the affected ORFs) within the insert or spanning all the junctions using the newly determined event sequence showed significant similarity with known allergens or toxins. Furthermore, sequence analysis using the updated A2704-12 event sequence did not reveal sufficient identity with microbial sequences (EFSA, 2017). Therefore, the updated A2704-12 sequence does not affect the likelihood of HGT. The complete updated bioinformatic analyses confirm all the previous conclusions for the A2704-12 soybean event.

The other studies performed for the risk assessment of GM soybean event A2704-12 are not affected by the new sequencing information.

On the basis of the available information, it cannot be confirmed whether the material used to produce the early generation soybean A2704-12 event sequence is exactly the same material used to determine the original soybean A2704-12 event sequence submitted in application EFSA-GMO-NL-2005-18 (T4 generation for the newly determined sequence and T3 or T4 generation for the originally reported sequence).

However, based on the analysis of the provided information including the 100% identity between the early generation (T4) and commercial variety GM soybean A2704-12 sequences as well as the 100% identity between the genomic flanking regions of those sequences and the corresponding regions of the non-GM soybean A2704 sequence, it can be concluded that it is highly unlikely that the reported sequence differences (including the single nucleotide difference in the 5′-chloroplast DNA fragment) are due to spontaneous mutations and these differences can therefore most likely be attributed to sequencing errors in the originally reported soybean A2704-12 event sequence.

4. Conclusions

Analysis of the information on the locations of the reported nucleotide differences for the soybean A2704-12 event indicated that out of the seven reported nucleotide differences, the six nucleotide differences in the 3′ soybean genomic flanking region and the single nucleotide in the 5′ -chloroplast DNA fragment, only the latter affects one of the newly created ORFs spanning the junction between the 5′-chloroplast DNA fragment and the insert. In addition, a single nucleotide located in the polylinker region of the insert and reported as ambiguous in the originally submitted A2704-12 event sequence is resolved in the new sequencing data. The ORF affected by the nucleotide difference in the 5′-chloroplast DNA fragment together with the six ORFs affected by the resolved base in the polylinker region were considered relevant for the current assessment and were therefore assessed. The complete bioinformatic analyses using the newly determined complete event sequence (including the affected ORFs) did not give rise to safety issues. Studies other than bioinformatics are not affected by this new sequence information.

Although it cannot be theoretically excluded, based on the analysis of the provided data, EFSA considers it highly unlikely that the reported sequence differences (including the single nucleotide difference in the 5′-chloroplast DNA fragment) are due to spontaneous mutations. Therefore, it can be concluded that the reported sequence differences can most likely be attributed to sequencing errors in the originally reported soybean A2704-12 event sequence. EFSA concludes that the original risk assessment of the GM soybean A2704-12 remains valid.

Documentation provided to EFSA

1) Letter from the European Commission received on 5 June 2018 concerning a request to analyse new sequencing information for GM soybean A2704-12.
2) Acknowledgement letter dated 29 June 2018 from EFSA to the European Commission.

3) Letter from applicant to EFSA received 10 July 2018 providing spontaneous additional information.
4) Letter from EFSA to applicant dated 19 July 2018 requesting additional information.
5) Letter from applicant to EFSA received on 31 July 2018 providing additional information.
6) Letter from EFSA to applicant dated 28 August 2018 requesting additional information.
7) Letter from applicant to EFSA received on 24 September 2018 providing additional information.

References

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EFSA (European Food Safety Authority), Gennaro A, Gomes A, Herman L, Nogue F, Papadopoulou N and Tebbe C, 2017. Technical report on the explanatory note on DNA sequence similarity searches in the context of the assessment of horizontal gene transfer from plants to microorganisms. EFSA supporting publication 2017:14 (7):EN-1273, 11 pp. https://doi.org/10.2903/sp.efsa.2017.EN-1273

Abbreviations

bp	base pair
GM	genetically modified
GMO	genetically modified organism
HGT	horizontal gene transfer
ORF	open reading frame
PAT	phosphinothricin acetyltransferase