Risk assessment of new sequencing data of GM maize event GA21

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STATEMENT

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Risk assessment of new sequencing data of GM maize event GA21

EFSA Panel on Genetically Modified Organisms (GMO)

Abstract

In 2007, 2010 and 2011, the European Food Safety Authority (EFSA) Panel on Genetically Modified Organisms (GMO Panel) concluded the assessment of genetically modified (GM) maize GA21, MIR604 × GA21 and MIR604 × GA21 × Bt11. These were found to be as safe as their conventional counterparts and other appropriate comparators with respect to potential effects on human and animal health and the environment. On 23 July 2015, the European Commission (EC) received from Syngenta new nucleic acid sequencing data on maize event GA21 and updated bioinformatic analyses using the new sequencing data. The EC tasked EFSA to analyse these data and to indicate whether the previous conclusions of the EFSA GMO Panel on the above-listed GM maizes remain valid. The EFSA GMO Panel used the appropriate principles described in its guidelines for the risk assessment of GM plants to analyse the received data. Compared with the sequencing data originally provided, the new sequencing data indicated a one-base pair addition in a non-coding region of the insert, a three-base pair deletion in the 3' flanking region of the insert, and a difference in the number of functional copies of the m*epsps* expression cassette. These differences were only recently identified, but it was confirmed that they had been present in the original plant material used for the risk assessment. Thus, with the exception of bioinformatics analyses, the studies performed for the risk assessment remain valid. The bioinformatic analyses performed on the new sequence did not give rise to safety issues. Therefore, the GMO Panel concludes that the original risk assessment of event GA21 as a single event, and as a part of stacked events, remains valid.

Keywords: GMO, maize (*Zea mays*), GA21, Regulation (EC) No 1829/2003

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1. Introduction

Genetically modified (GM) maize event GA21 was developed to confer tolerance to glyphosate (N-(phosphonomethyl)glycine)-based herbicides. This is achieved by the production of the mEPSPS (5-enolpyruvylshikimate-3-phosphate synthase) protein, which has a low binding affinity for glyphosate and maintains enzymatic activity in its presence.

The EFSA GMO Panel has previously assessed maize GA21 as a single event and as part of stacked events (see Table 1).

Table 1: EFSA GMO Panel scientific opinions on maize event GA21

Event	Application	EFSA Scientific Opinions
GA21	EFSA-GMO-UK-2005-19	EFSA, 2007
	EFSA-GMO-RX-GA21	
	EFSA-GMO-UK-2008-60	EFSA GMO Panel, 2011a
Bt11 × GA21	EFSA-GMO-UK-2007-48	EFSA GMO Panel, 2010a
Bt11 × MIR604 × GA21	EFSA-GMO-UK-2008-56	EFSA GMO Panel, 2010b

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

On 23 July 2015, Syngenta sent to the European Commission (EC) new sequencing information relating to maize events MIR604 and GA21, on the basis of Articles 9 and 21 of Regulation (EC) 1829/2003.

On 7 August 2015, the EC requested EFSA to analyse the data and analyses provided by Syngenta and indicate whether the conclusions of adopted opinion for maizes MIR604 and GA21 as single events or as part of stacked events, have to be adapted.

Subsequently, the EFSA GMO Panel evaluated the data and methodology provided for maize events MIR604 and GA21 and considered these elements in the context of previous conclusions.

The two maize events mentioned in the EC request, MIR604 and GA21, are addressed in independent EFSA GMO Panel statements. This statement addresses the data on maize event GA21.[1]

2. Methodologies and Data

2.1. Methodologies

The applicant followed the EFSA GMO Panel guidelines for the risk assessment of GM plants (EFSA GMO Panel, 2011b) to investigate the insert sequence and to perform the bioinformatic analyses.

In delivering this statement, the GMO Panel took into account the appropriate principles described in its guidelines for the risk assessment of GM plants (EFSA GMO Panel, 2011b).

2.2. Data

In delivering this statement, the EFSA GMO Panel took into account information provided by the applicant.

2.2.1. Sequence information previously submitted to EFSA for GA21

The applicant had previously submitted information on the sequence of maize event GA21, as part of application EFSA-GMO-UK-2005-19[2]; this information was updated in the context of application EFSA-GMO-UK-2008-60.[3]

[1] The EFSA GMO Panel statement on maize MIR604, is published in the EFSA Journal http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/4255.pdf
[2] Annex SSB-129-05 (confidential information).
[3] Annex SSB-129-05 A1 (confidential information).

Maize GA21 was reported to contain one insert that comprised three complete and three incomplete copies of the m*epsps* expression cassette,[4] as follows:

- Copy 1 contains an incomplete rice actin promoter, while the rest of the expression cassette is intact.
- Copies 2, 3 and 4 are complete.
- Copy 5 contains the rice actin promoter, first exon and intron, the optimised transit peptide and a fragment of the m*epsps* sequence; the *nos* terminator is lacking in this copy.
- Copy 6 contains only the rice actin promoter and a truncated actin first exon.

2.2.2. New sequence information for GA21 submitted as part of the current mandate

The applicant has recently re-sequenced the GA21 event in a stacked product, which revealed three discrepancies relative to the GA21 insert sequence recorded and reported in 2005 (EFSA-GMO-UK-2005-19). The differences are a nucleotide change in the actin promoter of copy 6, a three-base pair deletion within the 3' insert flanking region, and a difference in the number of complete m*epsps* cassettes present within the insert (see Table 2).

Table 2: Identified discrepancies in the sequence of the insert and flanking regions in maize GA21

Identified discrepancy	Reported in 2005	Reported in 2015
Actin promoter of copy 6	GTCGGATA	GTCGG**G**ATA
3' flanking region	GCCGCCTTT	GCC**---A**TT
Expression cassette copy number	3 complete and 3 truncated copies	2 complete and 3 truncated copies

To further investigate the origin of these differences, genomic DNA from the same GA21 material analysed in 2005 (EFSA-GMO-UK-2005-19) was used to re-sequence the GA21 insert and flanking regions.[5] The results indicated that the differences found in the stack were also present in the original GA21 material.

The updated length and structure of the insert were also confirmed by Southern blot analyses, by using a combination of four enzymes with the m*epsps*-specific probe.

The applicant carried out bioinformatic analyses using the updated nucleotide sequence in order to investigate (1) if any known maize genes were disrupted by the GA21 insert; (2) if the newly expressed protein or any other open reading frame (ORF) present within the insert and spanning the junction sites shows similarity to known allergens or toxins; and (3) if the flanking sequences and the insert contain sequences that would facilitate horizontal gene transfer to microorganisms.

3. Assessment of the potential consequences of the new sequence information

From the data provided by the applicant, it can be concluded that the sequence differences found in 2015 were already present in the original material used in the risk assessment process (EFSA GMO Panel, 2007; EFSA GMO Panel 2010a, b, 2011a). The data also show that the structure of the insert did not change and that the original material used for the risk assessment already contained two complete copies of the expression cassette, and not three, as erroneously reported in 2005.

The identified nucleotide sequence differences are located outside the coding sequence for the mEPSPS protein. Furthermore, the difference in the number of copies within the insert does not create

[4] The m*epsps* expression cassette contains the following elements: the 5' region of the actin 1 gene from rice, containing the promoter, first exon and intron; an optimised transit peptide constructed based on sequences from maize and sunflower ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo) genes; a modified maize *epsps* gene; and the 3' non-translated region from the *nos* gene from *Agrobacterium tumefaciens*.

[5] Additional information 11/09/2015.

new junction sequences. Therefore, with the exception of some aspects of the bioinformatic analyses, the studies performed for the risk assessment remain valid.

Considering the location of the identified nucleotides differences (in the 3' flanking region and in the actin promoter of copy 6), only the analysis of ORFs containing these differences with regard to potential similarity with allergens or toxins, as well as their implications on the potential for horizontal gene transfer, were considered relevant for the current assessment.

The search for similarity to allergens was performed using the (FAO/WHO) criterion of 35 % identity in a window of 80 amino acids. Results indicate that none of the ORFs containing the identified differences show similarity with known allergens or toxins.

Sequence analysis did not identify any similarity between the regions containing the identified differences and microbial sequences. Therefore, these differences do not affect the likelihood of HGT.

4. Conclusions

The EFSA GMO Panel was asked to evaluate the data provided by Syngenta for maize GA21 and to indicate whether its previous conclusions on this event remain valid.

The original material used in the risk assessment process already contained the differences reported in 2015. The change in the number of copies of the expression cassette actually present within the insert does not give rise to safety concerns. The nucleotide sequence differences are located outside the coding region of the insert and the bioinformatic analyses performed on the new sequence did not give rise to safety concerns. Therefore, the GMO Panel concludes that the original risk assessment of event GA21 as a single event, and as a part of stacked events, remains valid.

Documentation provided to EFSA

1. Letter from the European Commission, received on 7 August 2015, concerning a request to analyse new sequencing information of maize events MIR604 and GA21.

References

EFSA (European Food Safety Authority), 2007. Opinion of the Scientific Panel on Genetically Modified Organisms on applications (references EFSA-GMO-UK-2005-19 and EFSA-GMO-RX-GA21) for the placing on the market of glyphosate-tolerant genetically modified maize GA21, for food and feed uses, import and processing and for renewal of the authorisation of maize GA21 as existing product, both under Regulation (EC) No 1829/2003 from Syngenta Seeds S.A.S. on behalf of Syngenta Crop Protection AG. The EFSA Journal 2007, 541, 1–25.

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010a. Scientific Opinion on application (EFSA-GMO-UK-2007-48) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize MIR604 x GA21 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Syngenta Seeds. EFSA Journal 2010;8(5):1611, 30 pp. doi:10.2903/j.efsa.2010.1611

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010b. Scientific Opinion on application (EFSA-GMO-UK-2008-56) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize Bt11 x MIR604 x GA21 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Syngenta Seeds. EFSA Journal 2010;8(5):1616, 30 pp. doi:10.2903/j.efsa.2010.1616

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011a. Scientific Opinion on application (EFSAGMO-UK-2008-60) for placing on the market of genetically modified herbicide tolerant maize GA21 for food and feed uses, import, processing and cultivation under Regulation (EC) No 1829/2003 from Syngenta Seeds. EFSA Journal 2011;9(12):2480, 94 pp. doi:10.2903/j.efsa.2011.2480

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