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Risk assessment of new sequencing data on GM maize event MIR604

EFSA Panel on Genetically Modified Organisms (GMO)

Abstract

In 2009 and 2010, the EFSA GMO Panel concluded the assessment of genetically modified (GM) maize MIR604, MIR604 × GA21, MIR604 × Bt11 and MIR604 × GA21 × Bt11. These maize 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 MIR604 and updated bioinformatic analyses using the new sequencing data. EC tasked EFSA to analyse these data and to indicate whether the previous conclusions of the EFSA GMO Panel on the above-listed GM maize 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. The new sequencing data indicated a single base pair difference compared to the sequencing data originally provided, located in a non-coding region of the insert, which had already been present in the original plant material used for the risk assessment. Thus, with the exception of bioinformatic analyses, the studies performed for the risk assessment remain valid. The new sequencing data and 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 MIR604 as a single and as a part of stacked events remains valid.

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1. Introduction

Genetically modified (GM) maize event MIR604 was developed to confer resistance to the Western Corn rootworm (WCR) (*Diabrotica virgifera virgifera*) and other related coleopteran pests of maize like the Northern Corn rootworm (NCR) (*Diabrotica barberi*). This is achieved by the expression of a modified *cry3A* coding sequence (*mcry3A*) derived from *Bacillus thuringiensis* subsp. *tenebrionis* that encodes an insecticidally active mCry3A protein.

The EFSA GMO Panel has previously assessed maize MIR604 as a single event and as well as part of stacked events (see Table 1).

Table 1: EFSA GMO Panel scientific opinions on maize event MIR604.

Event	Application	EFSA Scientific Opinions
MIR604	EFSA-GMO-UK-2005-11	EFSA, 2009
MIR604 × GA21	EFSA-GMO-UK-2007-48	EFSA GMO Panel, 2010a
Bt11 × MIR604	EFSA-GMO-UK-2007-50	EFSA GMO Panel, 2010b
Bt11 × MIR604 × GA21	EFSA-GMO-UK-2008-56	EFSA GMO Panel, 2010c

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 evaluate the data and analyses provided by Syngenta and indicate whether, on the basis of these elements, the conclusions of adopted opinion for maize MIR604 and GA21 as single events or as part of stacked events, have to be adapted.

Subsequently, the EFSA GMO Panel has 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 MIR604.

2. Methodologies and Data

2.1. Methodologies

The applicant followed the EFSA 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, the GMO Panel took into account the appropriate principles described in its guidelines for the risk assessment of genetically modified (GM) plants (EFSA GMO Panel, 2011).

2.2. Data

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

2.2.1. Sequence information previously submitted to EFSA for MIR604

The applicant had previously submitted information on the sequence of maize event MIR604, as part of application EFSA-GMO-UK-2005-11.¹

Maize event MIR604 includes one insert comprising the *mcry3A* expression cassette, which contains the following elements: the MTL promoter of the metallothionein-like gene from *Zea mays*, the

¹ Technical dossier, Annex SSB-125-04 A1 (confidential information).

synthetic, maize-optimised *mCry3A* gene, based on the *cry3A* gene from *Bacillus thuringiensis* subsp. *tenebriosis*, and the terminator sequence of the nopaline synthase gene from *Agrobacterium tumefaciens*.

2.2.2. New information for MIR604 submitted as part of the current mandate

The sequencing of the MIR604 insert reported in 2004 was carried out on genomic DNA extracted from the single event. More recently, the applicant has re-sequenced the MIR604 insert in a stacked product, which revealed a one base pair (bp) discrepancy relative to the MIR604 insert sequence recorded and reported in 2004. The difference is located in the MTL promoter at nucleotide position 631 from the 5' end of the promoter (see Table 2).

Table 2: Identified discrepancy in the sequence of the maize MIR604 insert.

Identified discrepancy	Reported in 2004	Reported in 2015
MTL promoter	GAT <u>C</u> GTT	GAT <u>T</u> GTT

To further investigate this difference, genomic DNA from the same MIR604 material analysed in 2004 was used as template to amplify a 2.1 kb region of MIR604, where the difference was found. The results indicated that the one bp difference found in the stack was present in the original MIR604 material. The applicant assigned this difference to a reporting error in the original data set.

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 MIR604 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; 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 original material used in the risk assessment process (EFSA, 2009; EFSA GMO Panel 2010a, b, c) already contained the one bp difference found in 2015. Furthermore, the difference identified is located in the promoter region and does not affect the sequence of the newly expressed mCry3A protein. Therefore, with the exception of some aspects of the bioinformatics analyses, the studies performed for the risk assessment remain valid.

Considering the location of the one bp difference, only the analysis of ORFs containing the nucleotide difference with regard to potential similarity with allergens or toxins, as well as the implications of this difference on the potential for HGT 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 one bp change show similarity with known allergens or toxins.

Sequence analysis did not identify any similarity between the region containing the one bp difference and microbial sequences. Therefore, this nucleotide difference does not affect the likelihood of HGT.

4. Conclusions

The EFSA GMO Panel was asked to evaluate the data provided by Syngenta for maize MIR604 and to indicate whether its previous conclusions on this event remain valid.

The original material used in the risk assessment process already contained the one bp difference found in 2015, and the difference is present in a non-coding region of the insert and the bioinformatics 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 MIR604 as a single and as a part of stacked events remains valid.

Documentation provided to EFSA

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

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