EFSA STATEMENT

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Risk assessment of new sequencing information on genetically modified carnation FLO-40689-6

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Abstract

The GMO Panel has previously assessed genetically modified (GM) carnation FLO-40689-6 and concluded that there is no scientific reason to consider that the import, distribution and retailing in the EU of carnation FLO-40689-6 cut flowers for ornamental use will cause any adverse effects on human health or the environment. On 27 October 2017, the European Commission requested EFSA to analyse new nucleic acid sequencing data and updated bioinformatics data for carnation FLO-40689-6 and to indicate whether the conclusions of the GMO Panel on the previously assessed GM carnation FLO-40689-6 remain valid. The new sequencing data indicated the correction of one nucleotide compared to the sequencing data originally provided. The new sequence was corrected by removal of one nucleotide from the polylinker region in locus 1. The removal of this base pair reported in the new nucleic acid sequencing data for carnation FLO-40689-6 has been already 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 of GM carnation FLO-40689-6 remain valid. 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 carnation FLO-40689-6 remains valid.

Keywords: GMO, carnation, *Dianthus caryophyllus*, FLO-40689-6, nucleotide sequence, Directive 2001/18/EC

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1. Introduction

Genetically modified (GM) carnation FLO-40689-6 was obtained by *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*)-mediated transformation with plasmid pCGP1991.[1] The T-DNA region of the plasmid contains the flavonoid 3′,5′-hydroxylase (*f3′5′h*) and the dihydroflavonol-4-reductase (*dfr*) expression cassettes encoding for proteins that modify flower colour in the GM carnation, and the acetolactate synthase (*als*) cassette, conferring tolerance to sulfonylurea herbicides, used as a marker in the selection of transformants.

The GMO Panel has previously assessed carnation FLO-40689-6 as part of notification C/NL/06/01 (EFSA GMO Panel, 2008). This EFSA statement assesses the additional sequencing information received for the events in carnation FLO-40689-6.

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

On 16 February 2017, the European Commission received from Suntory Holding Limited new sequencing information relating to carnation FLO-40689-6. On 27 October 2017, the European Commission requested EFSA to evaluate the data and analyses provided by Suntory Holding Limited and indicate whether, on the basis of these elements, the conclusions of adopted opinion for carnation event FLO-40689-6 remain valid. Subsequently, EFSA has evaluated the data and methodology provided for carnation FLO-40689-6 and considered these elements in the context of the previous conclusion.

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 genetically modified (GM) plants (EFSA GMO Panel, 2011) and Regulation (EU) No 503/2013[2] 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), Regulation (EU) No 503/2013 and Directive 2001/18/EC.[3]

2.2.1. Sequence information previously submitted to EFSA for carnation FLO-40689-6

The applicant had previously submitted information on the sequence of carnation FLO-40689-6, as part of notification C/NL/06/01 (EFSA GMO Panel, 2008). Carnation FLO-40689-6 contains inserts in three loci, as described below:

- Locus 1: one copy of the T-DNA, containing the three expression cassettes;
- Locus 2: one insert containing the D8 terminator and the right T-DNA border, and an almost complete *f3′5′h* cassette (missing ca. 40 bp from the promoter);
- Locus 3: one incomplete copy of the *f3′5′h* cassette.

[1] T-DNA region of the pCGP1991 plasmid contains: the *dfr* cassette, encompassing the promoter, the *dfr* coding sequence and the terminator, cloned as a whole from the *Petunia* × *hybrida*; the *f3′5′h* cassette, containing the promoter sequence from *Antirrhinum majus* chalcone synthase (CHS) gene, the *f3′5′h* coding sequence from *Viola hortensis*, and the terminator sequence of the D8 gene encoding a *Petunia* × *hybrida* putative phospholipid transfer protein; and the *als* cassette consisting of the CaMV 35S promoter, the coding region and the terminator sequence from a mutated *als* gene from the SuRB locus of *Nicotiana tabacum*.

[2] 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 L 157, 8.6.2013, p. 1–48.

[3] 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.

2.2.2. New information for carnation FLO-40689-6 submitted as part of the current mandate

The applicant resequenced the carnation FLO-40689-6 event and compared this sequence with the originally submitted carnation FLO-40689-6 event sequence.[4] This revealed that the new sequence has one base pair less in the polylinker region in locus 1 compared to the original sequence (see Table 1).

Table 1: Identified differences in the sequence of the inserts and flanking regions in carnation FLO-40689-6

Identified difference	Position*	Original sequence	Updated sequence info
Polylinker (locus 1)	12995	GAGGGGGGGGCC	GAGGGGGGG-CC

*: Nucleotide position in the original sequence.

Genomic DNA used for the sequencing of the original GM carnation FLO-40689-6 event was isolated from plant material grown in Australia (2007 sequence information). However, both the original DNA and plant material were no longer available for an updated evaluation of this event sequence. Therefore, the plant material used to isolate genomic DNA for the resequencing of the GM carnation FLO-40689-6 event was grown at a different growth facility in Japan (2017 sequence information). To provide evidence that the difference between the original 2007 sequence and the newly submitted 2017 sequence found in locus 1 of event FLO-40689-6 can be attributed to sequencing error, the applicant resequenced the specific part of the locus in which the error was found using DNA extracted from plant material grown and independently propagated from the plants used to extract the DNA for the original application, at different geographic locations in Japan, Colombia and Ecuador (2018 sequence information). As it is very unlikely that spontaneous mutations would occur at exactly the same locus in independently grown plant material, and as the 2018 sequence was identical to the 2017 sequence, the applicant concluded that it is likely that the one nucleotide difference noted in the original 2007 sequence is due to sequencing error rather than mutation.[5]

The new 2017 sequence information obtained from loci 2 and 3 of GM carnation event FLO-40689-6 matched the originally submitted 2007 sequence.[4]

The applicant evaluated the impact of the nucleotide difference on the original bioinformatics analyses. Although the reported sequence change is in locus 1, the applicant provided bioinformatics analyses for all three loci of GM carnation event FLO-40689-6 using the corrected sequence for locus 1, and the original sequences of loci 2 and 3 in order to investigate if any open reading frame (ORF) present within the insert or spanning the junctions between the insert and genomic DNA shows similarity to known allergens or toxins.[6] In order to assess the possibility for horizontal gene transfer (HGT) by homologous recombination, the applicant performed a sequence identity analysis of the regions of bacterial origin for the complete corrected FLO-40689-6 event (3 loci).[7] In addition, and although the base pair change does not affect the flanking regions and the ORFs spanning the junction sites between insert and genomic DNA, the applicant provided new data on the possible interruption of endogenous plant genes.[8]

3. Assessment

The applicant resequenced the carnation FLO-40689-6 event and compared this sequence with the originally submitted carnation FLO-40689-6 event sequence, which revealed one base pair difference. The provided data indicated that the nucleotide difference in locus 1 of the GM carnation FLO-40689-6 event was likely already present in the originally submitted sequence (EFSA GMO Panel, 2008).

Bioinformatics analyses for the potential disruption of any known carnation genes was performed by the applicant, although the sequence difference does not affect the flanking regions and the ORFs spanning the junction sites between insert and genomic DNA. The data indicates that the insertion

[4] Info received on 09-11-2017 – Sequence analysis of the inserted transgenes and adjacent genomic DNA flanking regions in carnation GM-event FLO-40689-6; provision of complete and correct set of sequence data, 15-2-2017.
[5] Additional information received on 30-4-2018.
[6] Additional information C/NL/06/01 on 14-4-2017 and 30-4-2018.
[7] Additional information received on 09-7-2018.
[8] Additional information C/NL/06/01 on 14-4-2017, 30-4-2018 and 03-9-2018.

sites at loci 1 and 2 are located in a region with similarity to an unknown carnation gene. Based on sequence analysis of the carnation ORFs, there is a degree of homology to uncharacterised genes encoding RNase H domain-containing proteins from grape and *Arabidopsis*. The agronomic, phenotypic and compositional analyses of FLO-40689-6 which is assumed to be diploid and has only been vegetatively propagated 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 considered relevant for the current assessment. The bioinformatic searches for similarity to allergens were performed according to EFSA guidelines (EFSA GMO Panel, 2010, 2011) and the results confirmed previous assessments where no safety concerns were identified (EFSA GMO Panel, 2008, 2014). In addition, updated bioinformatics analyses of the newly created ORFs within the insert and at the junctions indicate that the expression of an ORF showing significant similarities to toxins or allergens is highly unlikely.

Bioinformatic analysis of event FLO-40689-6 revealed, for the three loci, DNA sequences of sufficient length and sequence identity for facilitating homologous recombination with bacterial DNA and thereby promoting HGT (EFSA, 2017). The bioinformatics analysis also revealed that the identified elements are separated by long non-homologous inserts that reduce the recombination efficiency (Kung et al., 2013). The three loci of carnation FLO-40689-6 do not include genetic elements which suggest that a selective advantage would be provided to bacterial recipients. EFSA concludes that the unlikely, but theoretically possible, horizontal transfer of recombinant genes from carnation FLO-40689-6 to bacteria does not raise any environmental safety concern.

The other studies performed for the risk assessment of GM carnation FLO-40689-6 are not affected by the new sequencing information.

4. Conclusions

Based on the analysis of the provided and additionally requested data, EFSA concludes that the original carnation material containing FLO-40689-6 event which was assessed previously (EFSA GMO Panel, 2008) already contained the single sequence difference reported by the applicant in 2017. The bioinformatics analyses 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 GM carnation FLO-40689-6 remains valid.

Documentation provided to EFSA

1) Letter from the European Commission dated 27 October 2017 concerning a request to analyse new sequencing information for carnation event FLO-40689-6.
2) Letter from EFSA to the European Commission dated 24 November 2017 acknowledging the reception of the request.
3) Letter from EFSA to the applicant dated 20 December 2017 requesting additional information.
4) E-mail from the applicant dated 11 January 2018 requesting a clarification teleconference.
5) Letter from EFSA dated 12 January 2018 inviting the applicant to a clarification tele-conference on 17 January 2018.
6) Email from EFSA to the applicant dated 17 January 2018 with the outcome of the clarification tele-conference.
7) Letter from the applicant to EFSA received 30 April 2018 submitting additional information requested.
8) Letter from EFSA to the applicant dated 4 May 2018 requesting additional information.
9) Letter from the applicant to EFSA received 14 May 2018 submitting additional information requested.
10) Letter from EFSA to the applicant dated 18 May 2018 requesting additional information.
11) Letter from the applicant to EFSA received on 09 July 2018 submitting additional information requested
12) Letter from EFSA to the applicant dated 23 July 2018 requesting additional information.
13) Letter from the applicant to EFSA received on 3 September 2018 submitting additional information requested.

References

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2008. Notification (Reference C/NL/06/01) for the placing on the market of the genetically modified carnation Moonaqua 123.8.12 with a modified colour, for import of cut flowers for ornamental use, under Part C of Directive 2001/18/EC from Florigene – Scientific Opinion of the Panel on Genetically Modified Organisms. EFSA Journal 2008;6(3):662, 25 pp. https://doi.org/10.2903/j.efsa.2008.662

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

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Abbreviations

als	acetolactate synthase
dfr	dihydroflavonol-4-reductase
f3′5′h	flavonoid 3′,5′-hydroxylase
GM	genetically modified
GMO	genetically modified organism
HGT	horizontal gene transfer
ORF	open reading frame