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Risk assessment of new sequencing information on genetically modified carnation FLO-40685-2

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Abstract

The GMO Panel has previously assessed genetically modified (GM) carnation FLO-40685-2 and concluded that there is no scientific reason to consider that the import, distribution and retailing in the EU of carnation FLO-40685-2 cut flowers for ornamental use will cause any adverse effects on human health or the environment. On 7 November 2017, European Commission requested EFSA to analyse new nucleic acid sequencing data and updated bioinformatics data for carnation FLO-40685-2 and to indicate whether the conclusions of the GMO Panel on the previously assessed GM carnation FLO-40685-2 remain valid. The new sequencing data indicated an additional three base pairs compared to the sequencing data originally provided: one base pair addition to the polyA tail of each of the two inserted flavonoid 3',5'-hydroxylase elements and one base pair addition to the sequence of one of the two D8 promoters in locus 1. These sequence differences are located outside the coding sequence for the newly expressed proteins and the base pairs described as differences in the new nucleic acid sequencing data for carnation FLO-40685-2 were reported to have 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-40685-2 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 carnation FLO-40685-2 remains valid.

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1. Introduction

Genetically modified (GM) carnation FLO-40685-2 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 dihydroflavonol 4-reductase (*df*r) and flavonoid 3',5'-hydroxylase (*f3',5'h*) expression cassettes, encoding for proteins that modify flower colour in the GM carnation, and the acetolactase 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-40685-2 as part of notification C/NL/13/02 (EFSA GMO Panel, 2016). This EFSA statement assesses the additional sequencing information received for the events in carnation FLO-40685-2.

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

On 17 October 2016, the European Commission (EC) received from Suntory Holding Limited new sequencing information related to carnation FLO-40685-2. On 7 November 2017, the EC 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-40685-2 remain valid. Subsequently, EFSA has evaluated the data and methodology provided for carnation FLO-40685-2 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 GM plants (EFSA GMO Panel, 2011) and Regulation (EU) No 503/2013² 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 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-40685-2

The applicant had previously submitted information on the sequence of carnation FLO-40685-2, as part of notification C/NL/13/02 (EFSA GMO Panel, 2016). Carnation FLO-40685-2 contains inserts in four loci, as described below:

- Locus 1: one copy of the T-DNA, containing the three expression cassettes and an incomplete copy of the T-DNA containing only the *f3',5'h* cassette with the right T-DNA border. The two T-DNA copies are separated by a carnation genomic DNA region;
- Locus 2: one insert containing the D8 terminator and the right T-DNA border;
- Locus 3: one complete and one incomplete copy of the *f3',5'h* cassette, containing both copies of D8 terminator sequences and the right T-DNA borders in a tail-to-tail orientation;
- Locus 4: an incomplete copy of the *als* cassette containing complete *als* gene, the CaMV 35S promoter and the left T-DNA border.

¹ T-DNA region of the pCGP1991 plasmid contains: the *df*r cassette, encompassing the promoter, the *df*r 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 L157, 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.

guidelines (EFSA GMO Panel, 2010, 2011). Results indicate that none of the newly generated ORFs show similarity with known toxins. However, two matches of 8 contiguous lysine (KKKKKKKK) or phenylalanine (FFFFFFFS) amino acids were identified when 3 of the 11 newly generated ORFs were used as query sequence for the allergen database. Analyses indicated that the expression of these ORFs, showing eight amino acids similarity to polyadenylation sequences of known allergens for the GM carnation FLO-40685-2 event, is highly unlikely.

Bioinformatic analysis⁹ of event FLO-40685-2 revealed, for the four loci, DNA sequences of sufficient length and sequence identity for facilitating homologous recombination with bacterial DNA and thereby promoting HGT (EFSA, 2017). These are in locus 1 the left and two right borders originating from an *Agrobacterium tumefaciens* octopine plasmid and the polylinker region. In locus 2, 3 and 4, the identified elements were not considered able to facilitate double homologous recombination. The bioinformatics analysis also revealed that the identified elements in locus 1 are separated by long non-homologous inserts (larger than 4.5 kbp), that reduce the recombination efficiency (Kung et al., 2013). The four loci of carnation FLO-40685-2 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-40685-2 to bacteria does not raise any environmental safety concern.

The other studies performed for the risk assessment of GM carnation FLO-40685-2 (EFSA GMO Panel, 2016) are not affected by the new sequencing information.

4. Conclusions

Based on an analysis of the provided and additionally data requested, EFSA concludes that the sequence of GM carnation FLO-40685-2 present in the original material used for the risk assessment process of the GM carnation FLO-40685-2 event already contained the nucleotide differences reported in 2016. 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-40685-2 remains valid.

Documentation provided to EFSA

- 1) Letter from the European Commission received on 7 November 2017 concerning a request to analyse new sequencing information for carnation FLO-40685-2.
- 2) Acknowledgement letter dated 24 November 2017 from EFSA to the European Commission.
- 3) Letter from EFSA to applicant dated 20 December 2017 requesting additional information.
- 4) 2018-01-12 E-mail from EFSA to applicant scheduling a clarification tele-conference.
- 5) 2018-01-17 E-mail EFSA to applicant providing the outcome of the clarification tele-conference.
- 6) Letter from applicant to EFSA received on 7 March 2018 providing additional information.
- 7) Letter from EFSA to applicant dated 12 April 2018 requesting additional information.
- 8) Letter from applicant to EFSA received on 7 May 2018 providing additional information.
- 9) Letter from EFSA to applicant dated 4 May 2018 requesting additional information.
- 10) Letter from applicant to EFSA received on 14 May 2018 providing additional information.
- 11) Letter from EFSA to applicant dated 18 May 2018 requesting additional information.
- 12) Letter from applicant to EFSA received on 12 June 2018 providing a timeline for responses.
- 13) Letter from applicant to EFSA received on 9 July 2018 providing additional information requested.

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), 2016. Scientific opinion on a Part C notification (reference C/NL/13/02) from Suntory Holdings Limited for the import, distribution and retailing of carnation FLO-40685-2 cut flowers with modified petal colour for ornamental use. EFSA Journal 2016;14(4):4431, 18 pp. <https://doi.org/10.2903/j.efsa.2016.4431>

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Kung SH, Retchless AC, Kwan JY and Almeida RP, 2013. Effects of DNA size on transformation and recombination efficiencies in *Xylella fastidiosa*. *Applied and Environmental Microbiology*, 79, 1712–1717.

Abbreviations

<i>als</i>	acetolactase synthase
<i>dfr</i>	dihydroflavonol 4-reductase
<i>f3',5'h</i>	flavonoid 3',5'-hydroxylase
GM	genetically modified
GMO	genetically modified organism
HGT	horizontal gene transfer
ORF	open reading frame