Vitenskapskomiteen for mat og miljø
Norwegian Scientific Committee for Food and Environment

VKM 2024

Assessment of new information in the renewal application (C/NL/09/02-001) for transgenic carnation event IFD-26407-2 for import as cut flowers for ornamental use in accordance with article 17 of Directive 2001/18/EC

This assessment is an update to the original VKM health and environmental risk assessment of transgenic carnation event IFD-26407-2 (VKM Report 2015: 18).

VKM 2024

Assessment of new information in the renewal application (C/NL/09/02-001) for transgenic carnation event IFD-26407-2 for import as cut flowers for ornamental use in accordance with article 17 of Directive 2001/18/EC. The notification is registered under notification number: C/NL/09/02-001 (ESFC number: GMHP-2024-23270).

This assessment is an update to the original VKM health and environmental risk assessment of transgenic carnation event IFD-26407-2 (VKM Report 2015: 18).

Norwegian Scientific Committee for Food and Environment (VKM)
Postboks 222 Skøyen
0213 Oslo
Norway

Phone: +47 21 62 28 00
Email: vkm@vkm.no

vkm.no

Cover photo: VKM Report 2015: 18

Table of Contents

Summary **4**
Sammendrag **5**
Background **6**
Excerpts of new information in the renewal application C/NL/09/02-001 ..... **7**
Conclusion **10**
References **11**

Summary

The Norwegian Environment Agency (NEA) has requested the Norwegian Scientific Committee for Food and Environment (VKM) to assess whether new scientific information provided in the renewal application (C/NL/09/02-001) for transgenic carnation event IFD-26407-2 changes VKMs previous conclusions in the risk assessment conducted during the original application (VKM Report 2015: 18).

Neither the various updated bioinformatic analyses provided in the application, nor the notifiers answers to requests for additional information from EU member states indicate an altered risk that would change the conclusions in VKMs previous risk assessment of transgenic carnation IFD-26407-2.

Sammendrag

Miljødirektoratet har bedt VKM om å vurdere hvorvidt ny vitenskapelig informasjon vedlagt i fornyelsessøknaden (C/NL/09/02-001) for transgen nellik IFD-26407-2 endrer konklusjonene i VKMs risikovurdering i forbindelse med den opprinnelige søknaden (VKM Report 2015: 18).

Hverken nye sekvensanalyser (databasesøk/bioinformatikkanalyser), eller søkers tilsvar på etterspurte data fra EU-medlemsland, indikerer en endret risiko som vil påvirke VKMs konklusjoner i den opprinnelige risikovurdering av transgen nellik IFD-26407-2.

Background

The Norwegian Environment Agency (NEA) has requested VKM to assess whether new scientific information provided in the renewal application (C/NL/09/02-001) for transgenic carnation event IFD-26407-2 changes VKMs previous conclusions in the risk assessment conducted during the original application (VKM Report 2015: 18).

Excerpts of new information in the renewal application C/NL/09/02-001

Flanking sequences

New information relating to flanking sequences of IFD-26407-2 was:

- Additional sequence information on the genomic DNA sequence flanking the inserted DNA in the event.
- Bioinformatic analysis of the additional genomic DNA sequence.
- Updated bioinformatic analysis of the inserted genes in IFD-26407-2.

Information on bioinformatic analysis of flanking sequences

Southern and sequencing analysis demonstrates that the transgenic carnation event IFD-26407-2 contains a single, intact, T-DNA insert (Nakamura et al., 2020). Longer flanking genomic DNA sequence of IFD-26407-2 has been obtained since the consent to marketing was provided for IFD-26407-2.

In the renewal application, bioinformatic analysis of the longer flanking sequences was conducted using two carnation genome databases to examine whether the location of the insert in the carnation genome could be determined. The two databases used were the DB carnation database developed by Yagi et al. (2014) and the carnation genome deposited at the NCBI-database by Zhang et al. (2022).

The longer flanking sequences that were generated were *2112 bp* at the left border and *901 bp* at the right border. Each of the two genomic DNA sequences flanking the T-DNA integration in IFD-26407-2 were subjected to blastn, tblastx and blastx.

- [*BLASTn: A nucleotide homology search for finding similar sequences in nucleotide databases by finding local regions of similarity.*
- *tBLASTx: A nucleotide homology search using both sequences of a query nucleotide sequence from all six possible open reading frames.*
- *BLASTx: An amino acid homology search using translated amino acid sequences from query nucleotide sequence*.]

Findings

The blastn and tblastx analyses identified the same scaffold sequences, the left border (LB) sequence is associated with scaffold 14540 and the right border (RB) with scaffold 1563. Highly significant similarities to scaffold sequences and linkage groups within the carnation nuclear genome databases were found for both LB and RB query sequences.

Further, using an ORF search, the sequence homology results suggest there was a possibility that the left border of the insert was inserted into a coding region of the carnation genome, Dca.21547, and the hit sequences are putative uncharacterized proteins. The homologous sites appeared in fragments, and the homology was not high. Therefore, it is highly unlikely that the flanking sequence of the left border encodes a functional protein. The LB and RB query sequences are associated with different linkage groups suggesting that the blast analysis was ineffective for locating the insert with a specific linkage group due to the genome quality. In addition, the ORF associated with the LB and RB were different which would not be expected if the T-DNA was inserted into a gene. Overall, the bioinformatic analysis did not provide evidence of which linkage group the insert might be located in and did not clearly indicate a gene in which the insert might be inserted.

Disruption of endogenous genes:

Open reading frames were generated from the longer flanking sequences using two ORF generation tools (EMBOSS and ORFfinder tools); one from stop codon to stop codon and one from start codon to stop codon. The ORF amino acid sequences were then used in a blastp (protein-protein BLAST) analysis (ORF analysis of flanking sequences CNL0901.pdf). A threshold limit of a minimum of 25 amino acids was set for selecting the ORFs for blastp.

The results obtained suggest that the left border flanking sequence against ORF sequences in a carnation genome database suggested a possibility that the left border of the T-DNA is inserted into a coding region, Dca.21547. However, comparison between the left border flanking sequence and the DNA sequence encoding the ORF Dca.21547 showed that the homologous sequences with Dca.21547 ORF found in the left border flanking sequence were limited to short fragmentary DNA sequences and did not cover the entire Dca.21547 ORF. This further suggests that the left border flanking sequence did not encode Dca.21547 ORF. Therefore, the results obtained suggest that the flanking sequences are unlikely to be part of a coding region.

Homology of ORF to toxin proteins: The UNIProtKB/Swissprot database was used to determine if the ORFs had significant homology to toxin proteins in the database.

Using query sequences from six ORFs, nine homology hits with an E-score of less than 1.0 were identified in the UniProtKB/Swiss-Prot database. The query sequences are unlikely to be homologous to toxin proteins because the lowest E-value score was too low to suggest a biologically significant homology and the Bit score for all hits was less than 40 suggesting no significant homology. There were no instances of 35% identity across the full sequence of the subject entry in the hit. None of the nine hits had the same entry identifier as the more than 10,000 toxin-related sequences in the database that was used for homology search.

Homology of ORF to allergen proteins: To determine the potential allergenicity, nine ORF query sequences were assessed using three on-line allergen databases (FAARP, COMPARE and Allermatch databases) using the full FASTA and 6/8mer exact match tools available within each database.

The results of the analysis indicate that none of the putative ORFs share significant homology to allergen proteins in the three up-to-date databases screened and no E-value scores less than 0.01

were observed (E-scores of less than 0.01 are indicative of homology while only those less than 1e-7 might suggest possible functional similarity). Further, the observed highest bit score was 26.7 suggesting that none of the hits obtained were of biological significance. Therefore, all nine ORF queries are unlikely to be allergens.

Updated bioinformatic analysis of the inserted genes in IFD-26407-2: Analysis of the sequences of the three newly expressed proteins (CYTB5, F3'5'H and ALS) in IFD-26407-2 using protein sequence databases indicated no biologically significant homology to toxins or allergens. The three newly expressed proteins are ubiquitous, well characterized proteins and are not known to be allergens.

Conclusion

The new information provided by bioinformatic analyses of longer flanking sequences was unable to determine whether the insert had disrupted an endogenous gene, and was unable to clearly determine the point of insertion in the carnation genome other than to suggest a non-coding region as the site of insertion. Bioinformatic analyses of all putative ORFs using protein sequence databases indicated no biologically significant homology to toxins or allergens. Further, analysis of the sequences of the three newly expressed proteins (CYTB5, F3’5’H and ALS) in IFD-26407-2 using protein sequence databases indicated no biologically significant homology to toxins or allergens.

VKM concludes that the new bioinformatic analyses of longer flanking sequences provided in the renewal application do not indicate an altered risk that would change the conclusions in VKMs previous risk assessment of transgenic carnation IFD-26407-2.

27.09.2024 Update

VKM has assessed the provided answers by the applicant to EU member state comments and/or requests. There is no new evidence in the new information (or answers) provided by the applicant about literature search strategy, toxin database bioinformatic review and toxicity assessment of whole plant extracts, that would change VKMs previous conclusions.

References

(VKM 2015) Final health and environmental risk assessment of genetically modified carnation Moonvelvet IFD-26407-2, VKM Report 2015: 18

Nakamura N, Suzuki T, Shinbo Y, Chandler S and Tanaka Y, 2020. Development of violet transgenic carnations and analysis of inserted transgenes. In: Onozaki T and Yagi M. (eds). The Carnation Genome, Compendium of Plant Genomes, Springer, Singapore, 135-146. doi:10.1007/978-981-15-8261-5_10

Yagi M, Kosugi S, Hirakawa H, Ohmiya A, Tanase K, Harada T, Kishimoto K, Nakayama M, Ichimura K, Onozaki T and Yamaguchi H, 2014. Sequence analysis of the genome of carnation (*Dianthus caryophyllus L*.). DNA Research, 21, 231-241. doi:10.1093/dnares/dst053

Zhang X, Lin S, Peng D, Wu Q, Liao X, Xiang K, Wang Z, Tembrock LR, Bendahmane M, Bao M and Wu Z, 2022. Integrated multi-omic data and analyses reveal the pathways underlying key ornamental traits in carnation flowers. Plant Biotechnology Journal, 20, 1182-1196. doi:10.1111/pbi.13801