



VKM 2024

Assessment of new information in the renewal application (C/NL/09/01-001) for transgenic carnation event IFD-25958-3 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-25958-3 (VKM Report 2015: 17).**

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**This assessment is an update to the original VKM health and environmental risk assessment of transgenic carnation event IFD-25958-3 (VKM Report 2015: 17).**

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# 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/09/01-001) for transgenic carnation event IFD-25958-3 changes VKMs previous conclusions in the risk assessment conducted during the original application (VKM Report 2015: 17).

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-25958-3.

# Sammendrag

Miljødirektoratet har bedt VKM om å vurdere hvorvidt ny vitenskapelig informasjon vedlagt i fornyelsessøknaden (C/NL/09/01-001) for transgen nellik IFD-25958-3 endrer konklusjonene i VKMs risikovurdering i forbindelse med den opprinnelige søknaden (VKM Report 2015: 17).

Hverken nye sekvensanalyser (databasesøk/bioinformatikkanalyser), eller søkers tilsvarende på etterspurt data fra EU-medlemsland, indikerer en endret risiko som vil påvirke VKMs konklusjoner i den opprinnelige risikovurdering av transgen nellik IFD-25958-3.

# Background

The Norwegian Environment Agency (NEA) has requested VKM to assess whether new scientific information provided in the renewal application (C/NL/09/01-001) for transgenic carnation event IFD-25958-3 changes VKMs previous conclusions in the risk assessment conducted during the original application (VKM Report 2015: 17).

# Excerpts of new information in the renewal application C/NL/09/01-001

## Flanking sequences

New information relating to flanking sequences of IFD-25958-3 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-25958-3.

## Information on bioinformatic analysis of flanking sequences

Southern and sequencing analysis demonstrates that the transgenic carnation event IFD-25958-3 contains a single, intact, T-DNA insert (Nakamura et al., 2020). Longer flanking genomic DNA sequence of IFD-25958-3 has been obtained since the consent to marketing was provided for IFD-25958-3.

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: 1077 bp at the left border and 1145 bp at the right border. Each of the two genomic DNA sequences flanking the T-DNA integration in IFD-25958-3 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 indicate that the insert is located in scaffold sequence 169 from the DB carnation genome. 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.14508, which putatively encodes a nucleoside triphosphate hydrolase protein. The identified homology appeared in fragments and restricted to only a third of the Dca.14508 translated ORF sequence. Overall, the bioinformatic analysis did not provide evidence of which linkage group the insert might be located 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. A threshold limit of a minimum of 25 amino acids was set for selecting the ORFs for blastp.

The results obtained suggest that the flanking sequences are unlikely to be part of a coding region. Comparison between the left border flanking sequence and the DNA sequence encoding the ORF Dca.14508.11 showed homologous sequences with Dca.14508.1 ORF found in the left border flanking sequence, and are limited to three divided sectors in the last third of the sequence of ORF14508.1. This further suggests that the left border flanking sequence is not the genomic DNA ORF Dca.14508.1. The ORF Dca.14508 was not associated with the RB indicating the T-DNA was not inserted into a gene. Furthermore, the ORFs associated with the LB and RB were different, which would not be expected if the T-DNA was inserted into a gene.

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 three 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. None of the nine hits had the same entry identifier as the more than 10,000 toxin-related toxin sequences in the database that was used for homology search.

Homology of ORF to allergen proteins: To determine the potential allergenicity, 12 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). No 6mer or 8mer matches were identified, suggesting a lack of significant homology to allergen proteins. Further, the observed highest bit score was 29.1 suggesting none of the hits obtained were of biological significance.



Updated bioinformatic analysis of the inserted genes in IFD-25958-3: Analysis of the sequences of the three newly expressed proteins (DFR, F3'5'H and ALS) in IFD-25958-3 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 about bioinformatic analysis from longer flanking sequences was unable to determine whether the insert had disrupted an endogenous gene, and did not provide clear evidence of as to which linkage group the insert might be located. Bioinformatic analysis 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 (DFR, F3'5'H and ALS) in IFD-25958-3 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-25958-3.

## **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, toxicity assessment of whole plant extracts and investigation of the expression level of a putative endogenous gene, a nucleoside triphosphate hydrolase protein, that would change VKMs previous conclusions.

# References

(VKM 2015) Final health and environmental risk assessment of genetically modified carnation Moonberry IFD-25958-3, [VKM Report 2015: 17](#)

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