Import and processing of genetically modified soybean DBN9004 with herbicide tolerance

COGEM advice CGM/210514-01

- The present application (EFSA/GMO/BE/2019/165) concerns the authorisation for import and processing for use in feed and food of genetically modified (GM) soybean DBN9004;
- The GM soybean expresses the genes *pat* and *cp4 epsps* conferring tolerance to glufosinate-ammonium and glyphosate containing herbicides, respectively;
- In the Netherlands, feral soybean populations do not occur and hybridisation of soybean with other species is not possible;
- The molecular characterisation of GM soybean DBN9004 meets the criteria of COGEM;
- There are no indications, nor there is reason to assume that the introduced traits will allow GM soybean DBN9004 to survive in the Dutch environment;
- COGEM is of the opinion that import and processing of GM soybean DBN9004 poses a negligible risk to the environment in the Netherlands;
- COGEM abstains from giving advice on the potential risks of incidental consumption since a food/feed assessment is carried out by other organisations.

1. Introduction

The present application (EFSA/GMO/BE/2019/165), filed by DaBeiNong Biotechnology Co. Ltd., concerns import and processing of genetically modified (GM) soybean DBN9004. This GM soybean line expresses the genes *pat* and *cp4 epsps*, conferring tolerance to glufosinate-ammonium and glyphosate containing herbicides.

2. Previous COGEM advice

COGEM has not previously advised on the import and processing of GM soybean lines expressing solely the combination of the *pat* and *cp4 epsps* genes. COGEM did advise positively on GM soybean lines expressing either the *pat* or (*cp4* or *2m*) *epsps* genes, or both genes in combination with a third herbicide tolerance-gene.[1,2,3,4]

3. Environmental risk assessment

3.1 Characteristics of soybean

Soybean (*Glycine max*) belongs to the *Leguminosae* (*Fabaceae*) family and is cultivated from equatorial to temperate zones. The optimum temperature for soybean growth is between 25°C and 30°C. Soybean is sensitive to frost and therefore does not survive freezing conditions.[5,6,7] In the Netherlands frost is common, with an average of 51 days a year of minimum temperatures below

0°C.[8] Although the Dutch climate is not optimal, soybean is cultivated on a small scale (about 476 acres in 2019 and 132 in 2020, according to provisional data).[9]

The soybean plant is not weedy in character.[6,7] To reduce yield losses during harvesting, soybean plants that have minimal seed scattering were selected for breeding. Soybean seeds rarely display dormancy, poorly survive in soil, and do not form a persistent soil seed bank.[6,10] Soybean volunteers are rarely observed throughout the world and do not effectively compete with other cultivated plants or primary colonisers.[6,7] In addition, volunteers are easily controlled mechanically or chemically.[7] Soybean volunteers are very uncommon in the Netherlands and have never resulted in establishment of wild populations.[11] To the best of COGEM's knowledge, there are no reports of feral soybean populations in Europe.

Soybean is predominantly a self-pollinating species. The anthers mature in the bud and directly pollinate the stigma of the same flower.[6,7] The cross-pollination rate of soybean is low and on average between 1 to 3%.[6,7,12,13,14,15,16] Soybean pollen disperses only over short distances. In Europe, hybridisation with other species is not possible because there are no wild relatives of soybean.[6,7]

Conclusion: In the Netherlands feral soybean populations do not occur and hybridisation of soybean with other species is not possible.

3.2 Description of the introduced genes and traits

DBN9004 was developed using *Agrobacterium tumefaciens* mediated transformation, the inserted genetic elements, and a description thereof, are listed in the table below. The list is limited to information on the introduced genes, corresponding traits, and regulatory elements.

Introduced genes	**Encoded proteins**	**Traits**	**Regulatory elements**
cp4 epsps	5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), codon-modified coding sequence from *Agrobacterium tumefaciens* strain CP4[17]	Tolerance to glyphosate	Promoter sequence GM17gTsf1 from *Glycine max*[18], sequence encoding signalling peptide AtCTP2 from *Arabidopsis thaliana*[19], terminator sequence PsE9 from *Pisum sativum*[20]
pat	Plant-optimised version of phosphinothricin acetyltransferase from *Streptomyces viridochromogenes*[21]	Tolerance to glufosinate-ammonium	35S promoter and 35S terminator sequence from *Cauliflower mosaic virus* (CaMV)[22,23]
For a detailed description of the introduced genes and traits, see references			

3.3 Molecular characterisation

DBN9004 was developed using *A. tumefaciens* mediated transformation with plasmid pDBN4003. This plasmid contains one T-DNA element consisting of a *cp4 epsps* expression cassette and a *pat* expression cassette. A single T-DNA copy is inserted into the nuclear genome, on chromosome 13, which was confirmed by Southern blot analysis. Southern blot analysis was also used by the applicant to demonstrate the absence of vector back-bone sequences in the genome. These results were complemented by DNA sequencing analysis of the T-DNA insert and the insert-to-plant genome junctions. The sequencing results confirmed the organisation of the elements within the insert and revealed an unintended deletion of 1545 bp at the position of the insert. According to the applicant, this deleted fragment was identified as an intergenic region and its removal did not disrupt any endogenous genes or annotated sequences.

As part of the bioinformatic data analysis, the entire insert as well as the flanking regions were screened, from stop codon to stop codon, for potential newly created Open Reading Frames (ORFs). The analysis indicated that one of the products of an identified ORF showed significant sequence similarity with a known protein of the GNAT family system, a toxin-antitoxin system. This putative ORF consists of most of the *pat* gene and part of its 35S promotor sequence. The identified GNAT family-component is a protein with N-acetyltransferase activity, similar to the phosphinothricin N-acetyltransferase encoded by the *pat* gene, and is not known to be associated with potential hazards. The bioinformatic analysis did not identify any other potential ORF-products with sequence similarities to known allergenic or toxic proteins. In conclusion, the molecular characterisation was conducted according to the criteria previously designed by COGEM.[24]

Conclusion: The molecular characterisation of soybean DBN9004 is adequate and no indications for potential environmental risks were identified.

3.4 Phenotypic and agronomic characteristics

The phenotypic and agronomic characteristics of soybean DBN9004 were analysed and compared to non-GM conventional soybean varieties. The characteristics of DBN9004 were found to be equivalent to the non-GM varieties, while taking into account natural variation, with the exception of the '1000 seeds weight'.

The weight of 1000 seeds of the DBN9004 soybean variant is shown to differ from the non-GM varieties, although statistical tests indicated that 'equivalence is more likely than not' (categorised according to the EFSA guidelines[25]). COGEM is of the opinion that a difference in seed weight is not considered to have an impact on the ability of soybean to survive or establish in the Dutch environment.

Additionally, the introduced traits do not give reason to assume that DBN9004 has an altered fitness compared to its conventional counterpart. COGEM is of the opinion that there are no indications that soybean DBN9004 will be able to survive or establish in the Dutch environment.

Conclusion: There are no indications that the introduced traits allow GM soybean DBN9004 to survive in the Netherlands. DBN9004 does not have an increased potential for the establishment of feral populations in the Netherlands.

4. Food/feed assessment

This application is submitted under Regulation (EC) 1829/2003, therefore a food/feed assessment is carried out by EFSA and national organisations involved in the assessment of food safety. In the Netherlands, WFSR carries out a food and/or feed assessment for Regulation (EC) 1829/2003 applications. The outcome of the assessment by other organisations (EFSA, WFSR) was not known when this advice was completed.

5. Post-market environmental monitoring (PMEM)

The applicant supplied a post-market environmental monitoring (PMEM) plan. The general surveillance (GS) plan differs in specific details, but is overall comparable to the GS plans from previous applications. In this GS plan, the applicant has not specified on the communication and agreements with third parties, but consults the same associations as stated in comparable GS plans. COGEM has published several recommendations for further improvement of the general surveillance (GS) plan[26,27] but considers the current GS plan adequate for import and processing of soybean DBN9004.

6. Overall conclusion

COGEM is of the opinion that import and processing of soybean DBN9004 poses a negligible risk to the environment in the Netherlands. COGEM abstains from giving advice on the potential risks of incidental consumption since other organisations carry out a food/feed assessment.

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