Import and processing of genetically modified soybean GMB151

COGEM advice CGM/190510-01

- The present application (EFSA/GMO/NL/2018/153) concerns the authorisation for import and processing for use in feed and food of genetically modified (GM) soybean GMB151;
- The GM soybean expresses the genes *cry14Ab-1.b* and *hppdPf-4Pa*, conferring resistance to soybean cyst nematode and tolerance to hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor 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 GMB151 meets the criteria of COGEM;
- There are no indications that the introduced traits alter the fitness of GM soybean GMB151;
- There is no reason to assume that the introduced traits will allow GM soybean GMB151 to survive in the Dutch environment;

- COGEM is of the opinion that import and processing of GM soybean GMB151 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/NL/2018/153), filed by BASF Agricultural Solutions Seeds US LLC, concerns import and processing of genetically modified (GM) soybean GMB151. This GM soybean line contains the genes *cry14Ab-1.b* and *hppdPf-4Pa*, conferring resistance to soybean cyst nematode and tolerance to hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor containing herbicides, such as isoxaflutole.

2. Previous COGEM advice

COGEM has not previously advised on import and processing of GM soybean GMB151. COGEM did advise positively on GM soybean line FG72 and on GM cotton line GHB811, which both contain the gene *hppdPfW336*.[1,2]

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.[3,4,5] In the Netherlands, frost is common. On average 58 days a year have minimum temperatures below 0°C.[6,7] Although the Dutch climate is not optimal, soybean is cultivated on a small scale (about 475 acres in 2018).[8]

The soybean plant is not weedy in character.[4,5] 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.[4,9] Soybean volunteers are rarely observed throughout the world and do not effectively compete with other cultivated plants or primary colonisers.[4,5] In addition, volunteers are easily controlled mechanically or chemically.[5] Soybean volunteers are very uncommon in the Netherlands and have never resulted in establishment of wild populations.[10] 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.[4,5] The cross-pollination rate of soybean is low and on average between 1 to 3%.[4,5,11,12,13,14,15] Pollen disperses only over short distances. In Europe, hybridisation with other species is not possible because there are no wild relatives of soybean.[4,5]

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

GMB151 was developed using *Agrobacterium tumefaciens* mediated transformation. A description of the inserted genetic elements is listed in the table below. The list is limited to information on the introduced genes, corresponding traits, and regulatory elements (promotors and terminators).

Introduced genes	**Encoded proteins**	**Traits**	**Regulatory elements**
cry14Ab-1.b	Cry14Ab-1 from *Bacillus thuringiensis*[16]	Resistance to soybean cyst nematode	Pubi10At promoter from *Arabidopsis thaliana* and 35S terminator from *Cauliflower mosaic virus* (CaMV)
hppdPf-4Pa	Modified 4-hydroxy-phenylpyruvate dioxygenase (HPPD-4) from *Pseudomonas fluorescens*[17]	Tolerance to hydroxy-phenylpyruvate dioxygenase inhibitor containing herbicides	Double enhanced promoter from CaMV and 3' untranslated region of the 35S transcript from CaMV
For a detailed description of the introduced genes and traits, see references			

3.3 Molecular characterisation

The applicant performed Next Generation Sequencing (NGS) and Junction Sequence Analysis (JSA). According to the applicant the results of these analyses show that the GMB151 soybean transgenic locus contains one copy of the T-DNA, comprising the complete *cry14Ab-1.b* gene cassette and the *hppdPf-4Pa* gene cassette which lacks the 5' part of the P2x35S promoter. GMB151 contains a 39 bp insert at the 3' end of the inserted T-DNA. 21 bp of this DNA show sequence identity to a region containing ORIpVS1 from the transforming plasmid pSZ8832 and 17 bp have sequence identity to the 3' flanking genomic sequence. Besides these 21 bp no backbone sequences are present in GMB151.

During the integration of the T-DNA, a fragment of 63 bp was deleted from the soybean genome. According to the applicant, the insertion of the T-DNA is located in the 3' untranslated region of a putative endogenous gene annotated as a BON1-associated protein 1-like protein. The applicant states that based on literature, the BON1-associated protein 1 has a function in a signal transduction cascade in *Arabidopsis thaliana*. At present, the biological function of the BON1-associated protein 1-like protein in soybean remains uncharacterized.

One base pair difference between the 3' flanking genomic sequence of the GMB151 soybean transgenic locus and the GMB151 insertion locus was found. According to the applicant, sequence differences between plants are commonly found and are due to natural genetic variation.[18]

The applicant screened the junctions between the T-DNA insert and the flanking soybean genomic DNA as well as the entire insert (from stop to stop codon) for potential newly created open reading frames (ORFs). According to the applicant, no significant sequence similarities between the putative products of these ORFs and no known or putative allergenic or toxic proteins were detected in bioinformatics analyses.

The molecular characterisation was conducted according to the criteria previously laid down by COGEM.[19]

Conclusion: The molecular characterisation of soybean GMB151 is adequate and no indications for potential environmental risks were identified.

3.4 Phenotypic and agronomic characteristics

The applicant analysed the phenotypic and agronomic characteristics of soybean GMB151 and noted that these, except for the introduced traits, are not different from the non-GM conventional counterpart, and are equivalent to the reference varieties, taking into account natural variation. The introduced traits do not give reason to assume that GMB151 has an altered fitness compared to conventional counterpart. COGEM is of the opinion that there are no indications that soybean GMB151 will be able to survive or establish in the Dutch environment.

Conclusion: There are no indications that the introduced traits allow the event GM soybean GMB151 to survive in the Netherlands. GMB151 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, RIKILT carries out a food and/or feed assessment for Regulation (EC) 1829/2003 applications. The outcome of the assessment by other organisations (EFSA, RIKILT) was not known when this advice was completed.

5. Post-market environmental monitoring (PMEM)

The applicant supplied a post-market environmental monitoring (PMEM) plan. COGEM has published several recommendations for further improvement of the general surveillance (GS) plan[20,21] but considers the current GS plan adequate for import and processing of soybean GMB151.

6. Overall conclusion

COGEM is of the opinion that import and processing of soybean GMB151 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.

References

1. COGEM (2012). Import of genetically modified soybean FG72 with glyphosate and isoxaflutole herbicide tolerance. COGEM advice CGM/120104-02
2. COGEM (2019). Import and processing of genetically modified cotton GHB811. COGEM advice CGM/190401-01
3. Bramlage WJ *et al*. (1978). Chilling stress soybeans during inhibition. Plant Physiol. 61: 525-529
4. Andersson MS & de Vicente MC (2010). Soybean (*Glycine max* (L.) Merr.). In: Gene flow between crops and their wild relatives. Eds: Andersson MS *et al*., The Johns Hopkins University Press, Baltimore
5. Organisation for Economic Co-operation and Development (OECD) (2000). Consensus document on the biology of *Glycine max* (L.) Merr. (Soybean)
6. Compendium voor de leefomgeving, meteorologische gegevens 1990-2015. www.compendiumvoordeleefomgeving.nl/indicatoren/nl0004-Meteorologische-gegevens-in--Nederland.html?i=9-54 (visited: April 11th, 2019) [In Dutch]
7. Koninklijk Nederlands Meteorologisch Instituut (KNMI). Klimaatatlas. www.klimaatatlas.nl/klimaatatlas.php?wel=temperatuur (visited: May 10th, 2019) [In Dutch]
8. Agrifirm. Sojateelt in Nederland. https://www.agrifirm.nl/contentassets/ba6c350248e847e99b5a5e41e5082301/ab-f012-0119nl_folder_soja_zoja_lr_web.pdf (visited April 11th, 2019) [In Dutch]

9. Organisation for Economic Co-operation and Development (OECD) (1993). Traditional crop breeding practices: An historical review to serve as baseline for assessing the role of modern biotechnology
10. FLORON Verspreidingsatlas Vaatplanten. *Glycine max* (L.) Merr. www.verspreidingsatlas.nl/1809 (visited: April 11th, 2019) [In Dutch]
11. Wang K & Li X (2013). Pollen dispersal of cultivated soybean into wild soybean under natural conditions. Crop Science 53: 2497-2505
12. Ahrent DK & Caviness CE (1994). Natural cross-pollination of twelve soybean cultivars in Arkansas. Crop Science Society of America 34: 376-378
13. Carlson JB & Lersten NR (1987). Reproductive morphology. In Soybeans improvement, production, and uses Second edition. Ed. Willcox JR, American Society of Agronomy, Madison
14. Ray JD *et al.* (2003). Soybean natural cross-pollination rates under field conditions. Environ. Biosafety Res. 2: 133-138
15. OECD (2008). Safety assessment of transgenic organisms. OECD consensus documents. Volume 1
16. Sanahuja G *et al.* (2011). *Bacillus thuringiensis*: a century of research, development and commercial applications. Plant Biotechnol. J. 9: 283-300
17. Boudec P *et al*. (2001). Mutated hydroxyphenylpyruvtate dioxybenase, DNA sequence and isolation of plants which contain such a gene and which are tolerant to herbicides. United States Patent
18. Zhu Y *et al.* (2003). Single-nucleotide polymorphisms in soybean. Genetics. 163: 1123-1134
19. COGEM (2014). Reconsideration of the molecular characterisation criteria for marketing authorisation of GM crops. COGEM policy report CGM/140929-02
20. COGEM (2010). General Surveillance. COGEM report CGM/100226-01
21. COGEM (2015). Advice on improving the general surveillance of GM crops. COGEM advice CGM/150601-02