Import of genetically modified soybean MON 87751 with two insect resistance traits

COGEM advice CGM/150327-01

Summary

The present application (EFSA/GMO/NL/2014/121) concerns import and processing for use in feed and food of the genetically modified soybean line MON 87751. Cultivation is not part of this application.

Soybean MON 87751 was generated by Agrobacterium *mediated transformation of conventional soybean and expresses the* cry1A.105 *and* cry2Ab2 *genes. As a result, soybean MON 87751 is resistant against certain lepidopteran insect pests.*

In Europe, there are no wild relatives of soybean and therefore, hybridisation with other species is not possible. Soybean does not possess any of the attributes commonly associated with problematic weeds such as seed shattering, dormancy or cold resistance. Establishment of feral soybean populations has never been observed in Europe.

The molecular characterisation of MON 87751 soybean meets the criteria of COGEM. COGEM considers the general surveillance plan adequate for import of MON 87751, although some aspects can be improved.

In conclusion, COGEM is of the opinion that import and processing of soybean line MON 87751 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 already carried out by other organisations.

Introduction

The scope of the present notification filed by Monsanto Company (EFSA/GMO/NL/2014/121), concerns import and processing of genetically modified (GM) soybean line MON 87751. This line was developed by *Agrobacterium tumefaciens* mediated transformation and expresses two versions of *cry* genes derived from *Bacillus thuringiensis*. As a result, soybean MON 87751 is resistant against certain lepidopteran insect pests.

Previous COGEM advices

In 2010 and 2014, COGEM advised positively on the import and processing of the insect resistant soybean lines MON 87701 and DAS-81419-2, respectively.[1,2] These lines produce several different Cry proteins. The encoding genes are different from the *cry* genes in MON 87751. In 2009, COGEM advised positively on the import and processing of the insect resistant maize line MON 89034.[3] The line produces two Cry proteins which are similar but not identical to the Cry proteins in soybean MON 87751 (97% and 99% amino acid identity).

Aspects of the crop

Soybean (*Glycine max*) is a member of the genus *Glycine* and belongs to the *Fabaceae* (*Leguminosae*) family. Soybean is grown from equatorial to temperate zones. The optimum temperature for soybean growth is between 25°C and 30°C. Soybean seeds will germinate at soil temperatures above 10°C. Under favourable conditions a seedling will emerge in a 5-7 day period. Soybean is sensitive to frost and therefore does not survive freezing conditions.[4,5,6]

In the Netherlands, frost is common. On average 58 days in a year have minimum temperatures below 0°C.[7,8] In summer days are long, whereas soybean is a quantitative short day plant.[5] The Dutch climate is therefore not optimal for cultivation of soybean. However, field trials with a number of soybean varieties have shown that cultivation of soybean under temperate climatic conditions is possible.[9,10,11] Further improvement of these varieties has resulted in soybean varieties more suited for cultivation in the Netherlands. In 2014 soybean was cultivated on a very small scale (approximately 100 acres).[9]

The soybean plant is not weedy in character.[5,6] Like for all domesticated crops, soybean has been selected for minimal seed scattering to reduce yield losses during harvesting. Soybean seeds rarely display dormancy, poorly survive in soil and do not form a persistent soil seed bank.[6,12] Soybean volunteers are rare throughout the world and do not effectively compete with other cultivated plants or primary colonisers.[5,6] In addition, volunteers are easily controlled mechanically or chemically.[5] To the best of COGEM's knowledge there exist no reports of feral soybean populations in Europe.

Soybean is predominantly a self-pollinating species.[6] The cross-pollination rate of soybean is an average of 1 to 3%.[5,10,13,14,15,16] The dispersal of pollen is limited because the anthers mature in the bud and directly pollinate the stigma of the same flower.[6] In Europe, hybridisation with other species is not possible because there are no wild relatives of soybean.[5]

Molecular characterisation

Soybean line MON 87751 was generated by transforming conventional soybean with the disarmed *Agrobacterium tumefaciens* strain AB30. The *Agrobacterium* strain carried a helper plasmid and the binary vector PV-GMIR13196. This vector contains two separate T-DNAs. The first T-DNA (T-DNA-I) contains the *cry1A.105* and *cry2Ab2* expression cassettes. The second T-DNA (T-DNA-II) contains two antibiotic resistance expression cassettes which were used as selectable markers. The transformation was followed by conventional breeding, segregation, selection and screening to isolate those plants that contain only T-DNA-I.

Characterisation of the DNA insert number in MON 87751 was conducted using a combination of Next Generation Sequencing technologies (NGS) and Junction Sequence Analysis (JSA).[17,18] In addition, directed sequencing (locus specific PCR on non-fragmented genomic DNA and sequence analyses) was used to verify the sequences of the DNA insert and the adjacent flanking DNA.

For NGS the genomic DNA from MON 87751 and its non-genetically modified counterpart soybean variety A3555, was fragmented and used to generate short (approximately 100 bp) randomly distributed sequenced fragments (sequencing reads). By using bioinformatic analysis tools, sequencing reads were selected whose alignments were 30 nucleotides or greater in length and displayed greater than or equal to 96.7% identity with either the sequence of a known single copy endogenous soybean gene, or the sequence of transformation plasmid PV-GMIR13196. Based on these data the coverage depth (the median number of times each base of the genome is independently sequenced) was determined to be ≥75×.

To determine the presence of backbone and T-DNA-II sequences and the number of insertions and T-DNA-I copy numbers, NGS/JSA was performed. NGS/JSA showed that soybean MON 87751 contains one copy of the T-DNA-I insert and that no plasmid backbone sequences or T-DNA-II sequences are present in the soybean genome. Analyses on genomic DNA of five successive generations of MON 87751 showed that the insert is stably inserted.

By ’directed sequencing’, the applicant determined the sequence of the insert, the sequence of the soybean genome insertion site, and 1,334 bp of the 5’ flanking region and 1,187 bp of the 3’ flanking region of the insert. The analysis showed that the inserted T-DNA-I is identical to the T-DNA-I of plasmid PV-GMIR13196, and that the insert is flanked by soybean genomic DNA. In addition, 1 bp was inserted at the insertion site and 7 bp of the soybean genomic DNA were deleted. Furthermore, a 16 bp sequence was integrated in the 5’ flanking region upstream of the insert.

An overview of the T-DNA-I introduced in MON 87751 (10,119 basepairs (bp)) is given below:

- Border region containing the right border sequence used for transfer of the T-DNA-I
- Intervening sequence used in DNA cloning
- P-*Act2* (promoter, leader and intron sequences from the *act2* gene of *Arabidopsis thaliana*, that directs transcription in plant cells)
- Intervening sequence
- TS-*CTP2* (targeting sequence of the *ShkG* gene from *A. thaliana* encoding the EPSPS transit peptide region that directs transport of the protein to the chloroplast)
- Intervening sequence
- CS-*cry2Ab2* (codon optimized coding sequence for the Cry2Ab2 protein of *B. thuringiensis* that provides insect resistance)
- Intervening sequence
- T-*Mt* (3’UTR sequence from *Oryza sativa Mt* gene encoding a metallothionein like protein that directs polyadenylation of mRNA)
- Intervening sequence
- P-*RbcS4* (promoter and leader sequences from *A. thaliana rbcS* gene family encoding small subunit *ats1A* that directs transcription in plant cells)
- TS-*RbcS4* (targeting sequence from *A. thaliana rbcS* gene family encoding small subunit *ats1A* that directs transport of the protein to the chloroplast)
- CS-*cry1A.105* (codon optimized coding sequences of the Cry1Ab, Cry1F and Cry1Ac proteins of *B. thuringiensis*, to produce a chimeric protein that provides insect resistance)

- Intervening sequence
- T-*Pt1* (3'UTR sequence from *Medicago trunculata PT1* gene encoding a phosphate transporter that directs polyadenylation of mRNA
- Intervening sequence
- Border region containing the left border sequence used for transfer of the T-DNA-I

The applicant performed bioinformatic analyses using different software tools on the region that was deleted during the integration of the insert in the soybean genome. According to the applicant the analyses indicate that it is unlikely that endogenous open reading frames (ORFs) at the insertion site were disrupted.

The applicant bioinformatically analysed the junctions between the insert and the soybean genome from stop to stop codon for the presence of ORFs. A total of 12 putative polypeptides of eight amino acids or greater in length were evaluated for potential sequence similarities to known allergens, toxins and biologically active proteins. According to the applicant the results of these analyses demonstrated no similarities.

Bioinformatical analysis on the putative peptides encoded by translation of reading frames 1 through 6 of the T-DNA-I insert did not demonstrate similarities to known allergens, toxins and biologically active proteins that could effect human or animal health, apart from the introduced *cry1A.105* and *cry2Ab2* genes, which are toxic for certain insects.

In summary, MON 87751 soybean contains a single intact T-DNA insert in its nuclear genome. A 16 bp sequence is inserted in the 5' flanking region of the insert. At the insertion site 7 bp are deleted and 1 bp is inserted. No backbone sequences are present in the plant genome. Bioinformatic analysis of ORFs spanning the insert-genome junctions and within the insert, identified no significant amino acid sequence similarities to known allergens, toxins and biologically active proteins harmful to humans or animals. The molecular characterisation of soybean MON 87751 meets the criteria laid down by COGEM.[19]

Properties of the introduced genes conferring insect resistance

Soybean line MON 87751 contains the *cry1.105* and *cry2Ab2* genes. These *cry* genes encode delta-endotoxins, which have a primary effect against certain lepidopteran insects and provide protection from feeding damage caused by insect pests.

Delta-endotoxins are solubilised in the midgut of susceptible insects and are activated by midgut proteases to release a toxin fragment. This toxin fragment binds to specific receptors on the epithelial surface of the midgut, which causes pores to open. This leads to disruption of the movement of solutes across the gut epithelium and allows gut bacteria to escape the midgut and enter the hemolymph where they cause septicaemia and death.[20,21]

Environmental risk assessment

The current notification concerns import and processing of soybean line MON 87751. In case of spillage, MON 87751 soybean seed may be released into the environment. Soybean seeds rarely display dormancy, poorly survive in soil and do not survive freezing winter conditions. In summer, days are long whereas soybean is a quantitative short day plant. In summary, the Dutch climatic conditions are not optimal for growth of soybean.

Soybean volunteers are rare throughout the world and do not effectively compete with other cultivated plants, weeds or primary colonisers.[5,6] In addition, volunteers are easily controlled mechanically or chemically.[5] There are no indications that soybean line MON 87751 has an increased potential to survive or establish feral populations in case of incidental spillage.

In conclusion, COGEM is of the opinion that soybean line MON 87751 is not able to establish itself and form feral populations in the Netherlands.

Food/ feed assessment

COGEM abstains from giving advice on the potential risks of incidental consumption since a food/feed assessment is already carried out by other organisations.[22] 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, a food and/or feed assessment for Regulation (EC) 1829/2003 applications is carried out by RIKILT. Regarding the risks for food and feed, the outcome of the assessment by other organisations (EFSA, RIKILT) was not known when this advice was completed.

General surveillance

General surveillance (GS) has been introduced to be able to observe unexpected adverse effects of GM crops on the environment. A GS plan is required for every application for market authorisation.

The current GS plan states that unanticipated adverse effects will be monitored by existing monitoring systems, which include the authorisation holder and operators involved in the handling and use of viable soybean. The third parties (operators) involved in GS will report adverse effects to the authorisation holder.

In 2010, COGEM published a report on the principles that, according to COGEM, should be followed for general surveillance.[23] COGEM considers the submitted GS plan adequate for import of MON 87751 however, the plan could be improved on the following points.

In the present GS plan, the authorisation holder states that the operators have agreed to provide information relevant to the monitoring of MON 87751 to the authorisation holder. The GS plan could be improved by a guarantee that operators will monitor for unanticipated effects. In particular a statement is lacking that the authorisation holder will give evidence that the operators collect this information.

The GS plan further states that, if the authorisation holder identifies an unexpected adverse effect caused by the GM plant, he will inform the European Commission immediately. COGEM is of the opinion that Member States should also be directly informed of these effects by the authorisation holder, to ensure that appropriate measures for protection of humans and the environment can be implemented immediately.

In the EFSA guidance document, EFSA states that the applicant should make raw data and analysis of monitoring data available to the Competent Authorities and the European Commission.[24] COGEM agrees with this request and points out that the applicant should include a statement on this point in the GS plan.[25]

Considerations and advice

COGEM has been asked to advise on import and processing for use in food and feed of soybean line MON 87751. Soybean line MON 87751 contains the *cry1.105* and *cry2Ab2* genes conferring resistance to certain lepidopteran insect pests.

Although it is possible to cultivate soybean varieties in the Netherlands, cultivation takes place on a very small scale. The Dutch climate is not optimal for soybean growth. Soybean volunteers are rare throughout the world and do not effectively compete with other cultivated plants or primary colonisers. Modern soybean cultivars do not possess any of the characteristics commonly associated with problematic weeds. In addition, establishment of feral soybean populations in Europe has never been observed. There are no indications that expression of the introduced *cry1.105* and *cry2Ab2* genes will increase the potential of soybean to establish feral populations.

COGEM is of the opinion that the risk of spread of soybean MON 87751 within the Netherlands due to incidental spillage of this soybean line is negligible. Wild relatives of soybean are not present in Europe and therefore introgression of the inserted genes into closely related species cannot occur. The molecular characterisation of MON 87751 soybean meets the criteria of COGEM. Although the GS plan could be improved, COGEM considers the submitted GS plan adequate for import of MON 87751.

Based on the aspects discussed, COGEM is of the opinion that import and processing of soybean MON 87751 poses a negligible risk to the environment in the Netherlands. A food/feed safety assessment is carried out by other organisations. Therefore, COGEM abstains from advice on the potential risks of incidental consumption.

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