Import and processing of insect resistant soybean line MON87701

COGEM advice CGM/100810-01

Summary

The present application of Monsanto Company (file EFSA/GMO/BE/2010/79) concerns the import and processing for use in feed and food of genetically modified soybean line MON87701. Cultivation is not part of this application.

Soybean line MON87701 was developed through Agrobacterium tumefaciens mediated transformation. The soybean line expresses the cry1Ac gene derived from Bacillus thuringiensis, conferring resistance to certain lepidopteran insects.

The applicant confirmed by Southern blot analyses that one intact copy of the insert was integrated at a single insertion locus in the genome of MON87701 and that the plasmid's backbone, used for transformation, was absent in MON87701. Sequence analysis spanning the 5' and 3' junctions of the insertion site and the genomic DNA indicated a 32 bp deletion as well as a 14 bp insertion at the 5'end of the insert in MON87701. Furthermore, several sequences of eight amino acids or greater in length spanning the 5' junction and 3' junction of the insert and the genomic DNA were identified. No sequence similarities between any known toxins or allergens were demonstrated. In the opinion of COGEM, the molecular analysis of MON87701 has been adequately performed.

In Europe, closely related species of soybean are not present and soybean does not possess any of the attributes commonly associated with problematic weeds. Besides, establishment of feral soybean populations has never been observed in Europe. Hybridization with other species is not possible because there are no closely related species of soybean present. Due to the climatic and geographical conditions, survival of soybean is not possible in the Netherlands. Because there is no reason to assume that the inserted genes would introduce or increase the potential for soybean to establish feral populations, COGEM is of the opinion that incidental spillage of soybean will not pose a risk to the environment.

In conclusion, COGEM is of the opinion that import and processing of soybean line MON87701 poses a negligible risk to the environment and has no objections against an authorization for import and processing of MON87701. COGEM points out that a food/feed safety assessment is carried out by other organizations. Therefore, COGEM abstains from advice on the potential risks of incidental consumption.

Introduction

The present application (file EFSA/GMO/BE/2010/79) by Monsanto Europe S.A., concerns the import and processing of genetically modified soybean MON87701. This soybean line was

produced by *A. tumefaciens* mediated transformation of conventional soybean and expresses the *cry1Ac* gene derived from *B. thuringiensis* subsp. *kurstaki* conferring resistance to certain lepidopteran insect pests.

Previous COGEM advice

In February 2010 COGEM advised positively on import and processing of hybrid line MON87701 x MON89788.[1] In addition to the *cry1Ac* gene, this hybrid line contains the *cp4 epsps* gene, resulting in tolerance to glyphosate containing herbicides.

The application for hybrid line MON87701 x MON89788 included specific data for MON87701, needed in support of the safety assessment and authorization of the hybrid line. For the present application, the applicant provided additional information with respect to the molecular characterisation of MON87701, including an overview of the predicted and inserted fragments in table format and an amino acid sequence alignment comparing the Cry1Ac protein in MON87701 and in *B. thuringiensis*.

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. Due to the climatic and geographical conditions, cultivation of soybean is not possible in the Netherlands. The optimum temperature for soybean growth is between 25°C and 30°C. In the Netherlands, 16.6°C was the average summer temperature from 1971 to 2009. The average temperature of the three warmest summers since 1901 was 18.6°C.[2] In addition, soybean does not survive freezing. In the Netherlands frost is common; during winter on average 38 days are measured with a minimum temperature below 0°C.[2] Moreover, during the Dutch growth season the days are long, whereas soybean is a quantitative short-day plant that needs short days for induction of flowering.

Soybean is predominantly a self-pollinating species. The cross-pollination rate of soybean is less than 1%.[3] Cross-pollination occurs by insects. The dispersal of pollen is limited because the anthers mature in the bud and directly pollinate the stigma of the same flower.[4] Therefore, insect-born export of pollen is limited.[3] In Europe, hybridization with other species is not possible because there are no closely related species of soybean.

The soybean plant is not weedy in character.[4] Cultivated soybean rarely displays dormancy[4] and seeds of cultivated soybean survive poorly in soil.[5] Soybean volunteers are rare and do not effectively compete with other cultivated plants or primary colonizers.[4] In addition, volunteers are easily controlled mechanically or chemically.[4] Establishment of feral soybean populations has never been observed in Europe.

Molecular characterization

Overview of the construction and inserted genetic elements of parental soybean line MON87701
The genetically modified soybean line MON87701 was produced by *Agrobacterium tumefaciens*-mediated transformation using the binary transformation plasmid PV-GMIR9. PV-GMIR9 contains two transfer-DNAs (T-DNAs), T-DNA I with the trait of interest (*cry1Ac*) and T-DNA II encoding a selectable marker (*cp4 epsps*). Following selection of the transformants, the inserted T-DNA II was segregated from progeny through subsequent traditional breeding and genetic selection processes. The inserted T-DNA I containing the *cry1Ac* gene was maintained. The result is a soybean containing only the *cry1Ac* expression cassette. An overview of the introduced T-DNA I sequences is given below:

- Sequence flanking 5′ end of the insert. Soybean nuclear genomic DNA.
- B-right border. DNA region from *A. tumefaciens* containing the right border sequence used for transfer of the T-DNA.
- IS. Intervening sequence used in DNA cloning.
- *P^7-RbcS4*. Promoter, leader, and 5' non-translated region of the *Arabidopsis thaliana RbcS4* gene encoding ribulose 1,5-bisphosphate carboxylase small subunit 1A. Promoter is active in above ground tissues.
- *TS-CTP1*. Targeting sequence encoding the transit peptide of the Arabidopsis *RbcS4* encoding small subunit 1A transit peptide from *A. thaliana*, present to direct the Cry1Ac protein to the chloroplast.
- *CS-cry1Ac*. Codon-modified coding sequence of the Cry1Ac protein of *B. thuringiensis.*
- IS. Intervening sequences used in DNA cloning.
- *T-7S α'*. 3' region of the *Sphas1* gene of soybean encoding the 7*S α'* seed storage protein β-conglycinin, including 35 nucleotides of the carboxyl terminal β-conglycinin coding region with the termination codon and the polyadenylation sequence. The element functions to terminate translation and direct polyadenylation of the mRNA.
- IS. Intervening sequence used in DNA cloning.
- B-Left Border. DNA region from *A. tumefaciens* containing the left border sequence used for transfer of the T-DNA.
- Sequence flanking 3' end of the insert. Soybean genomic DNA.

Properties of the genes introduced in MON87701
The *cry1Ac* gene encodes a δ-endotoxin which specifically acts against insects of the order of Lepidoptera. These toxins are solubilized in the midgut of susceptible insects and are activated by midgut proteases to release a toxin fragment. The toxin fragment binds to specific receptors on the

midgut epithelium. Subsequently, pores are formed in the membranes of the gut cells of the insect, enabling midgut bacteria to enter the body cavity, which leads to septicemia and death.[6]

Molecular analysis MON87701

The applicant showed by Southern blot analyses that one intact copy of the T-DNA I containing the *cry1Ac* expression cassette was integrated at a single integration locus in the genome of MON87701. Furthermore, the applicant demonstrated by hybridization analysis that the backbone of plasmid PV-GMIR9 and the T-DNA II, harboring the *cp4 epsps* expression cassette, was absent in MON87701. Results obtained by PCR amplification and DNA sequence analyses confirmed that an intact insert was integrated. Sequence analyses spanning the 5' and 3' junctions of the insertion site and the genomic DNA indicated a 32 bp deletion of genomic DNA as well as a 14 bp insertion at the 5'end of the insert in MON87701. Bioinformatic analysis by BLASTn and BLASTx searches (GenBank databases, June 2009) indicate that it is unlikely that endogenous soybean open reading frames were disrupted by the T-DNA I insertion. Furthermore, DNA sequences spanning the 5' and 3' junctions of the MON87701 insertion site and the genomic DNA were analyzed from stop codon to stop codon. Ten sequences of eight amino acids or greater in length spanning the 5' junction and 3' junction of the insert and the genomic DNA were identified. Bioinformatic analyses were performed by alignment searches in allergen, toxin and protein databases (AD_2009, TOX_2009 and PRT_2009) respectively. The results of these analyses demonstrated no sequence similarities between any known toxins or allergens and the ten putative polypeptides.

COGEM is of the opinion that the molecular characterization of MON87701 has been adequately performed and meets the criteria laid down by COGEM.[7] These criteria match the EFSA criteria.[8,9]

Environmental risk assessment

The current application of soybean line MON87701 concerns import and processing. In case of spillage, soybean seed may be released into the environment. Due to the climatic and geographical conditions, cultivation of soybean is impossible in the Netherlands as soybean is a quantitative short-day plant that needs short days for induction of flowering. The optimum temperature for growth is between 25°C and 30°C, and soybean does not survive freezing. The introduced trait does not increase the ability of soybean seed to survive in the Dutch climatic conditions. In view of the above, there are no reasons to assume that the expression of *cry1Ac* gene increases the potential of MON87701 to establish feral populations in case of incidental spillage.

Since 2008 COGEM abstains from giving advice on the potential risks of incidental consumption in case a food/feed assessment is already carried out by other organizations.[10] This application is

submitted under Regulation (EC) 1829/2003, therefore a food/feed assessment is carried out by EFSA. Other organizations who advice the competent authorities can perform an additional assessment on food safety although this is not obligatory. In the Netherlands a food and/or feed assessment for Regulation (EC) 1829/2003 applications is carried out by RIKILT.

General surveillance plan

General surveillance (GS) has been introduced to be able to observe unexpected adverse effects of GM crops on the environment. The setting or population in which these effects might occur is either not, or hardly predictable.

The GS plan states that unanticipated adverse effects will be monitored by existing monitoring systems which include the authorization holder and operators involved in the handling and use of viable MON87701 soybean. Recently, COGEM formulated criteria for GS plans concerning applications for import and cultivation of GM crops.[11]Although the GS plan could be improved by a guarantee that operators will monitor for unanticipated effects, COGEM considers the current GS plan sufficient for import and processing of MON87701 soybean.

Advice

COGEM has been asked to advice on import and processing for use in food and feed of soybean line MON87701. COGEM is of the opinion that incidental spillage of soybean leading to the spread of soybean within the Netherlands is negligible. Establishment of feral soybean populations in European countries has never been observed and there is no reason to assume that the presence and expression of the introduced genes increases the potential of soybean to establish feral populations. In addition, closely related species of soybean are not present in Europe and therefore introgression of the inserted genes into closely related species cannot occur. In the opinion of the COGEM, the molecular analysis of MON87701 is adequate.

Based on the considerations put forward in this advice, COGEM is of the opinion that import and processing of soybean MON87701 poses a negligible risk to the environment.

References

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