Import and processing of genetically modified soybean MON87769 expressing two desaturase genes

COGEM advice CGM/100414-01

Summary

The present application of Monsanto Company (file EFSA/GMO/UK/2009/76) concerns the import and processing for use in feed and food of soybean line MON87769. Cultivation is not part of this application.

Soybean line MON87769 was obtained by Agrobacterium tumefaciens mediated transformation. The soybean expresses the Pj.D6D gene derived from Primula juliae and Nc.Fad3 derived from Neurospora crassa resulting in the production of stearidonic acid (SDA), an omega-3 fatty acid.

The applicant showed by Southern blot analyses that one copy of the insert was integrated at a single integration locus in the genome of MON87769 and that the backbone of the plasmid used for transformation was absent in MON87769. Sequence analyses spanning the 5' and 3' junctions of the insertion site and the genomic DNA indicated a 9 bp deletion of genomic DNA and two small insertions of 17 and 8 bp. No endogenous soybean open reading frames (ORFs) were disrupted. Sequence analyses also identified several sequences of eight amino acids or greater in length. No sequence similarities between any known toxins or allergens were demonstrated. In the opinion of COGEM, the molecular analysis of MON87769 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 MON87769 poses a negligible risk to the environment and has no objections against an authorization for import and processing of MON87769. 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/UK/2009/76) by Monsanto Europe S.A., concerns the import and processing of genetically modified soybean MON87769. Soybean MON87769 was produced by *Agrobacterium tumefaciens* mediated transformation of conventional soybean and expresses the *Pj.D6D* gene derived from *Primula juliae* and *Nc.Fad3* derived from *Neurospora*

crassa. The introduction of these genes results in the seed specific production of stearidonic acid (SDA), an omega-3 fatty acid.

Previous COGEM advice

To date, COGEM has not advised on soybean MON87769 or the specific genes introduced in this soybean. However, in 2006 COGEM advised positively on the import and processing of soybean 305423 expressing a *gm-fad2-1* gene fragment resulting in a high oleic phenotype.[1] Also in 2008 COGEM advised positively on the import and processing of hybrid soybean line 305423 x 40-3-2. In addition to a high oleic phenotype, this hybrid line is tolerant to glyphosate-containing herbicides due to the presence of the *cp4 epsps* gene.[2]

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 impossible 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.[3] 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.[3] 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%.[4] 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. Therefore, insect-born exportation of pollen is limited.[4] 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.[5] Cultivated soybean rarely displays dormancy[5] and seeds of cultivated soybean survive poorly in soil.[6] Soybean volunteers are rare and do not effectively compete with other cultivated plants or primary colonizers.[5] In addition, volunteers are easily controlled mechanically or chemically.[5] Establishment of feral soybean populations has never been observed in Europe.

Molecular characterisation

Soy line MON87769 was produced by *A. tumefaciens* mediated transformation of meristematic soybean tissue. This soy line expresses the *Pj.D6D* gene derived from *P. juliae* and *Nc.Fad3* derived from *N. crassa* resulting in the production of stearidonic acid (SDA), an omega-3 fatty acid.

Overview of the construction and inserted genetic elements of parental soybean line MON87769
The binary transformation plasmid PV-GMPQ1972 was used to produce the genetically modified soybean line MON87769. PV-GMPQ1972 contains two separate transfer-DNA's (T-DNA's). T-DNA I contains the desaturase genes *Pj.D6D* and *Nc.Fad3* and T-DNA II harbours 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 desaturase genes was maintained. The result is a soybean containing only the *Pj.D6D* and *NcFAD3* expression cassettes. An overview of the introduced T-DNA I sequences is given below:

- *Sequence flanking 5' end of the insert*. *G. max* genomic DNA.
- *B-Right border.* 42 bp of the DNA region from *A. tumefaciens* containing the right border sequence used for transfer of the T-DNA.
- *P-7Sα'*. Seed-specific promoter and leader sequence from the *Sphas 1* gene of *G. max.*
- *CS-Pj.D6D.* Coding sequence for the fatty acid delta-6 desaturase from *P. juliae.*
- *T-tml.* 3' non-translated region of the *tml* gene from *A. tumefaciens* octopine-type Ti plasmid.
- *P-7Sα.* Promotor and leader sequence from the *Sphas2* gene from *G. max*.
- *CS-Nc.Fad3.* Codon optimised coding sequence for the gene from *N. crassa* encoding delta-15 desaturase.
- *T-E9. 3'* non-translated region of the *Pisum sativum rbcS2* gene which functions to direct polyadenylation of the mRNA.
- *B-Left Border.* 273bp of the DNA region from *A. tumefaciens* containing the left border sequence used for transfer of the T-DNA.
- *Sequence flanking 5' end of the insert*. *G. max* genomic DNA.

Properties of the genes introduced in MON87769
The genetically modified soybean line MON87769 contains the *P. juliae Pj.D6D* gene and the *N. crassa Nc.Fad3 gene*. The introduction of these genes results in the seed-specific production of the PjΔ6 en NcΔ15 desaturase proteins.

Δ6 desaturase is responsible for the conversion of α-linolenic acid (ALA) into stearidonic acid (SDA). SDA is an omega-3 fatty acid which is a normal metabolic precursor to the longchain, poly-unsaturated omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in humans and animals. Longchain poly-unsaturated omega-3 fatty acids have been linked to reductions in cardiovascular disease, cancer, inflammation and neurological disorders.[7] Δ6 desaturase may also convert linoleic acid (LA) into γ–linolenic acid (GLA). The addition of Δ15 desaturase increases ALA levels, allowing greater flux to SDA accumulation in MON87769. It also lowers the LA levels (by conversion of LA into ALA) and hence lowers the substrate pool for GLA production.

Molecular analysis MON87769

The applicant showed by Southern blot analyses that one intact copy of the T-DNA I containing the *Pj.D6D* and *NcFAD3* expression cassettes was integrated at a single integration locus in the genome of MON87769. Furthermore, the applicant demonstrated by Southern blot analysis that the backbone of plasmid PV-GMPQ1972 and the T-DNA II, harbouring the *cp4 epsps* expression cassette, was absent in MON87769. 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 9 bp deletion of genomic DNA and two small insertions of 17 and 8 bp. 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 MON87769 insertion site and the genomic DNA were analyzed from stop codon to stop codon. Five sequences of eight amino acids or greater in length spanning the 5' junction and six sequences of eight amino acids or greater in length spanning the 3' junction 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 eleven putative polypeptides.

COGEM is of the opinion that the molecular characterization of MON87769 has been adequately performed and meets the criteria laid down by COGEM.[8] These criteria match the EFSA criteria.[9,10]

Environmental risk assessment

The current application of soybean line MON87769 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 traits do 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 the *Pj.D6D* and *Nc.Fad3* genes increases the potential of MON87769 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.[11] 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. Regarding the risks for food and feed, the outcome of the assessment by other organizations (EFSA, RIKILT) was not known at the moment of completion of this advice.

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 MON87769 soybean. Recently, COGEM formulated criteria on which GS plans concerning Dutch applications for import and cultivation of GM crops have to comply.[12] 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 MON87769 soybean.

Advice

COGEM has been asked to advice on import and processing for use in food and feed of soybean line MON87769. 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.

The molecular analysis of MON87769 is adequate. Based on the aspects discussed, COGEM is of the opinion that import and processing of soybean MON87769 poses negligible risks to the environment.

References

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