Import and processing of genetically modified soybean MON87701 x MON89788

COGEM advice CGM/100202-01

Summary

The present application of Monsanto Company (file EFSA/GMO/NL/2009/73) concerns the import and processing for use in feed and food of soybean line MON87701 x MON89788. Cultivation is not part of this application.

Soybean line MON87701 x MON89788 was obtained by conventional crossbreeding of the two parental lines MON87701 and MON89788. The hybrid line contains the cry1Ac gene, conferring resistance to certain lepidopteran insects, and the cp4 epsps gene, resulting in tolerance to glyphosate containing herbicides. In the opinion of COGEM, the molecular analysis of soybean line MON87701 x MON89788 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 x MON89788 pose a negligible risk to the environment and has no objections against an authorization for import and processing of MON87701 x MON89788. 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

Present application (file EFSA/GMO/NL/2009/73) by Monsanto Europe S.A., concerns the import and processing of genetically modified soybean MON87701 x MON89788. This hybrid line expresses the *cry1Ac* gene derived from *Bacillus thuringiensis* subsp. *kurstaki* conferring resistance to certain lepidopteran insect pests. Additionally, the hybrid line expresses the *cp4 epsps* gene derived from *Agrobacterium* sp. strain CP4, conferring tolerance to glyphosate containing herbicides.

MON87701 x MON89788 soybean was produced by conventional crossbreeding of two genetically modified parental lines. Parental line MON89788 has an EU approval for import, food and feed.[1] In Canada and the United States MON89788 has been legalized for use in food and

feed, and for environmental release.[2] Besides, MON89788 has been authorized for commercial import.[3] Parental line MON87701 has never been assessed by the EU or EFSA.

Previous COGEM advice

Initially COGEM advised negatively on import and processing of parental soybean MON89788[4] because its molecular characterization did not meet the criteria laid down by COGEM.[5] Furthermore, COGEM questioned the general surveillance plan. After the applicant provided additional information on the molecular characterization and the general surveillance plan, COGEM advised positively.[6] COGEM has not advised on MON87701 and the hybrid line yet.

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.[7] 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.[7] 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%.[8] 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.[9] Therefore, insect-born exportation of pollen is limited.[8] 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.[9] Cultivated soybean rarely displays dormancy[9] and seeds of cultivated soybean survive poorly in soil.[10] Soybean volunteers are rare and do not effectively compete with other cultivated plants or primary colonizers.[9] In addition, volunteers are easily controlled mechanically or chemically.[9] Establishment of feral soybean populations has never been observed in Europe.

Molecular characterization

MON87701 x MON89788 was produced by crossing the two genetically modified parental soybean lines MON87701 and MON89788 using traditional breeding methods. The hybrid soybean line contains the *cry1Ac* gene, derived from parental soybean line MON87701, and the *cp4 epsps* gene, derived from parental soybean line MON89788.

The molecular characterization of soybean MON89788 was previously evaluated in 2007 and 2008,[4,6] the molecular characterization of soybean line MON87701 has not been evaluated by

COGEM before. Therefore, this advice concerns the evaluation of both parental line MON87701 and hybrid line MON87701 x MON89788.

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 contained two transfer-DNA's (T-DNA's), 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.
- 45 bp remaining DNA region from the Right Border region derived from the PV-GMIR9 plasmid of *A. tumefaciens*. The Right Border region is used for transfer of the T- DNA.
- IS. Intervening sequence used in DNA cloning.
- P-*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 transcription and direct polyadenylation of the mRNA.
- IS. Intervening sequence used in DNA cloning.
- B-Left Border. 264 bp DNA region from the Left Border region remaining after integration.
- Sequence flanking 3' end of the insert. Soybean genomic DNA.

Overview of the construction and inserted genetic elements of parental soybean line MON89788
The genetically modified soybean line MON879788 was produced by *A. tumefaciens*- mediated transformation using the binary transformation plasmid PV-GMGOX20. PV-GMGOX20 contained T-DNA which hosted the *cp4 epsps* expression cassette. An overview of the T-DNA sequences introduced 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 sequences used in DNA cloning.
- P–*FMV/Tsf1*. Chimeric promoter consisting of enhancer sequences from the *35S* promoter of the *Figwort mosaic virus* and the promoter from the *Tsf1* gene of *A. thaliana* encoding the elongation factor EF-1 alpha.
- L–*Tsf1*. 5' Nontranslated leader (exon 1) from the *Tsf1* gene of *A. thaliana* encoding the elongation factor EF-1 alpha.
- I–*Tsf1* 2. Intron from the *Tsf1* gene of *A. thaliana* encoding the elongation factor EF-1 alpha.
- IS. Intervening sequences used in DNA cloning.
- TS–*CTP2*. Sequences encoding the chloroplast transit peptide from the *ShkG* gene of *A. thaliana* encoding EPSPS.
- CS– *cp4 epsps*. Codon optimized coding sequence of the *aroA* (*epsps*) gene from the *Agrobacterium* sp. strain CP4 encoding the CP4 EPSPS protein.
- IS. Intervening sequences used in DNA cloning.
- T–*E9*. 3' Nontranslated sequence from the ribulose-1,5- bisphosphate carboxylase small subunit (*RbcS2*) *E9* gene of pea (*Pisum sativum*).
- IS. Intervening sequences 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 nuclear genomic DNA.

Properties of the genes introduced in MON87701 x MON89034

MON87701 x MON89788 was produced by crossbreeding of the two genetically modified parental soybean lines MON87701 and MON89788. The hybrid soybean line contains the *cry1Ac* gene, derived from parental soybean line MON87701, and the *cp4 epsps* gene, derived from parental soybean line MON89788.

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.[11]

The *cp4 esps* gene encodes the CP4 EPSPS protein. EPSPS is a natural occurring enzyme involved in the biosynthesis of aromatic amino acids. Glyphosate inhibits EPSPS, resulting in a lack of amino acids essential for growth and development of plants. The CP4 EPSPS protein is not inhibited by glyphosate which results in plants tolerant to glyphosate containing herbicides. [12]

Molecular analysis MON87701

The applicant confirmed 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. Additionally, hybridization analyses showed that the insert is stable over several generations. 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. Results obtained by PCR amplification and DNA sequence analyses confirmed that an intact insert was integrated. Sequence comparison between the pre-insertion site and the MON87701 flanking regions indicated a 32 bp deletion 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) showed that no 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 coding for eight amino acids or greater spanning the 5' junction and 3' junction of the insert and the genomic DNA were identified and bioinformatic analyses were performed making use of the AD_2009, TOX_2009 and PRT_2009 databases. Results of these analyses demonstrated no structurally-relevant similarities between any known toxins or allergens and the ten putative polypeptides.

In conclusion, in COGEM's opinion the molecular characterization of MON87701 has been sufficiently performed and meets the criteria laid down by COGEM.[5]

Updated bioinformatic analysis MON89788

In 2008 COGEM gave a positive advice on import and processing of parental soybean MON89788.[6] Its characterization met COGEM's criteria and was judged to be adequate.[5] Sequences spanning the 5' and 3' junctions between the insert and the genomic DNA were translated in silico from stop to stop codon in all frames. None of the putative polypeptides showed homology to known toxins and allergens. The applicant performed a renewed bioinformatics analysis of the MON89788 flanking sequences on updated GenBank databases (June 2009) which confirmed the earlier findings.

Molecular analysis MON87701 x MON89788

Genomic DNA from MON87701 × MON89788, MON87701, MON89788 and conventional soybean varieties was digested with restriction enzymes and subjected to Southern blot analysis. The applicant confirmed that the hybrid line contained the unaltered copies of both MON87701 and MON89788.

In conclusion, in COGEM's opinion the molecular characterization of MON87701 x MON89788 has sufficiently been performed and meets the criteria laid down by COGEM.[5]

Environmental risk assessment

The current application of soybean line MON87701 x MON89788 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 *cry1aC* and *cp4 epsps* genes increases the potential of MON87701 x MON89788 to establish feral populations in case of incidental spillage.

Since 2008 COGEM abstains from giving advices on the potential risks of incidental consumption in case a food/feed assessment is already carried out by other organizations.[13] 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 the completion of this advice.

General surveillance plan

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

The general surveillance 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 x MON89788 soybean. Although the general surveillance plan could be improved by a guarantee that operators will monitor for unanticipated effects, COGEM considers the general surveillance plan sufficient for import and processing of MON87701 x MON89788 soybean.

Advice

COGEM has been asked to advice on import and processing for use in food and feed of soybean line MON87701 x MON89788. The North-Western European climate prohibits survival and establishment of soybean. Furthermore, modern soybean cultivars do not possess any of the characteristics commonly associated with problematic weeds and there is no reason to assume that presence and expression of the introduced gene increases the potential of soybean to establish feral populations. In addition, establishment of feral soybean populations in European countries has never been observed. COGEM is of the opinion that incidental spillage of soybean is very unlikely to lead to the spread of soybean within the Netherlands. In addition, closely related species of soybean are not present in Europe and therefore introgression of the inserted genes into closely related species can not occur.

The molecular analysis of MON87701 and MON87701 x MON89788 is adequate.

Based on the aspects discussed, COGEM is of the opinion that import and processing of soybean MON87701 x MON89788 pose a negligible risk to the environment.

Additional remark

The applicant showed that the hybrid line contained intact copies of both the insertion cassettes. The results were confirmed by Southern blot analysis using genomic digested DNA from MON87701, MON89788, MON87701 x MON89788, and conventional soybean varieties. The obtained blots are presented in the Technical Dossier and a supporting study.

Strikingly, the quality of the blots shown in the study the applicant refers to is lower than the quality of the blots shown in the Technical Dossier. This can lead to unnecessary confusion in the risk assessment. COGEM points out that data presented by the applicant have to be unambiguous.

References

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