Import of genetically modified soybean MON87708 with a new herbicide tolerance trait

COGEM advice CGM/110801-02

Summary

The present application of Monsanto Europe S.A. (EFSA/GMO/NL/2011/93) concerns the import and processing for use in feed and food of the genetically modified soybean line MON87708. Cultivation is not part of this application.

Soybean line MON87708 was obtained by Agrobacterium-mediated transformation of conventional soybean. The line expresses the dmo *gene from* Stenotrophomonas maltophilia*, conferring tolerance to dicamba containing herbicides.*

In Europe, closely related species of soybean are not present and therefore, hybridization with other species is not possible. Soybean does not possess any of the attributes commonly associated with problematic weeds and establishment of feral soybean populations has never been observed in Europe. Due to the climatic and geographical conditions, survival of soybean is not possible in the Netherlands. There is no reason to assume that the introduced trait would introduce the potential for soybean to establish feral populations. COGEM is of the opinion that the risk of spread of soybean MON87708 within the Netherlands due to incidental spillage of this soybean is negligible.

In the opinion of COGEM, the molecular analysis of soybean line MON87708 is sufficient. Besides the normal DMO protein expressed in MON87708, an alternatively processed longer version of the protein containing the N-terminal sequence of the pea Rubisco small subunit protein is present in MON87708. There is no indication that the longer DMO protein or the normal DMO protein will pose a risk to the environment.

Although the general surveillance (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 soybean line MON87708.

In conclusion, COGEM is of the opinion that import and processing of soybean line MON87708 poses a negligible risk to the environment. COGEM points out that a food/feed safety assessment is carried out by other organisations. Therefore, COGEM abstains from advice on the potential risks of incidental consumption.

Introduction

The present application by Monsanto Europe S.A. (EFSA/GMO/NL/2011/93), concerns the import and processing of genetically modified soybean MON87708. The line confers tolerance to the dicamba containing herbicides.

COGEM has not advised on dicamba tolerant plants before. Dicamba tolerant soybean line MON87708 is not yet authorized for cultivation in any country, though notifications have been submitted in the USA.

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 measured in De Bilt. The average temperature of the three warmest summers in De Bilt since 1901 was 18.6°C.[1] In addition, soybean does not survive freezing.[2] In the Netherlands frost is common; during winter on average 38 days are measured with a minimum temperature below 0°C.[1] 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.[2]

Soybean is predominantly a self-pollinating species. The cross-pollination rate of soybean is less than 1%.[2] The dispersal of pollen is limited because the anthers mature in the bud and directly pollinate the stigma of the same flower.[2] In Europe, hybridization with other species is not possible because there are no closely related species of soybean.[2]

The soybean plant is not weedy in character.[2] Cultivated soybean seeds rarely display dormancy and poorly survive in soil.[3] Soybean volunteers are rare and do not effectively compete with other cultivated plants or primary colonizers.[2] In addition, volunteers are easily controlled mechanically or chemically.[2] Establishment of feral soybean populations has never been observed in Europe.

Soybean is grown primarily for the production of beans, has a multitude of uses in the food and industrial sectors and represents one of the major sources of edible vegetable oil and proteins for livestock feed use.[2] Today, soybean is the most prevalently grown oilseed in the world, with approximately 222 million metric tons produced in 2007, which represented 56% of world oilseed production that year.[4] Soybean is grown as a commercial crop in over 35 countries. The major producers of soybean are the US, Brazil, Argentina and China.[5]

Molecular characterization

MON87708 was developed by *Agrobacterium*-mediated transformation of conventional soybean. Two expression cassettes were initially introduced in MON87708. One cassette contains the *dmo* gene from the bacteria *Stenotrophomonas maltophilia*, conferring tolerance to dicamba. The other cassette expresses the *cp4 epsps* gene derived from *Agrobacterium* sp., conferring tolerance to glyphosate containing herbicides.[6] Glyphosate tolerance was used as a tool to select transformed plants. This cassette was subsequently removed from the line by conventional breeding and selection, resulting in MON87708 soybean which is tolerant to dicamba herbicides, but sensitive to herbicides containing glyphosate.[7]

Soybean MON87708 was produced by *Agrobacterium tumefaciens* mediated transformation of meristematic tissue of soybean line A3525 using the binary vector PV-GMHT4355. PV-GMHT4355 contains two transfer DNA regions (T-DNAs). T-DNA I contains the *dmo* gene expression cassette. T-DNA II contains the *cp4 epsps* expression cassette for glyphosate tolerance, but as mentioned, this cassette was removed from the line by breeding and selection.

Properties of the initially introduced gene cassettes
The *dmo* gene which is introduced in MON87708 encodes a dicamba mono-oxygenase enzyme (DMO) which demethylates dicamba, rendering it inactive. As a result MON87708 is tolerant to treatment with dicamba containing herbicides.

Overview of the inserted genetic elements of MON87708
An overview of the insert introduced in MON87708 and its flanking regions is given below:

- Right Border. 52 bp sequence containing the Right Border region from *A. tumefaciens* used for transfer of the T-DNA.
- Intervening sequence. Sequence used in DNA cloning.
- *PC1SV* promoter. Promoter from the Full-Length Transcript gene of *Peanut chlorotic streak virus* that directs transcription in plant cells.
- Intervening sequence. Sequence used in DNA cloning.
- *TEV* leader. 5' non-translated region from the *Tobacco etch virus* genome, involved in regulating gene expression.
- Intervening sequence. Sequence used in DNA cloning.
- *RbcS* chloroplast targeting sequence. Two sequences from the pea *Rubisco small subunit* gene; the chloroplast transit peptide sequence and the N-terminal sequence of the Rubisco small subunit protein, encoding 24 amino acids of the mature protein. These sequences direct transport of the DMO precursor protein to the chloroplast.
- Intervening sequence. Sequence used in DNA cloning.
- Modified *dmo* gene. Coding sequence of the dicamba mono-oxygenase (DMO) enzyme derived from *S. maltophilia*. One amino acid was added for cloning purposes, and one amino acid was changed.
- Intervening sequence. Sequence used in DNA cloning.
- *E9* terminator. 3' Untranslated region of the pea *RbcS2* gene which functions to direct polyadenylation of the mRNA.
- Intervening sequence. Sequence used in DNA cloning.
- Left Border. 253 bp sequence containing the Left Border region from *A. tumefaciens* used for transfer of the T-DNA.

Expressed proteins
Dicamba mono-oxygenase is isolated from the bacteria *S. maltophilia*. The MON87708 DMO protein diverges from the *S. maltophilia* DMO protein by two amino acids. One alanine was added in position two for cloning purposes and a tryptophan to cysteine substitution occurred at position 112 during PCR amplification of the *dmo* gene from *S. maltophilia*. These changes do not affect the catalytic site of the enzyme, where dicamba is bound.

The MON87708 *dmo* gene sequence encodes a 39.8 kDa DMO protein. Alternative processing of the precursor protein in the chloroplast results in a second, functional DMO protein with a molecular weight of 42 kDa. This protein has 27 additional amino acids at its N-terminus, of which 24 amino acids originate from the N-terminus of the pea Rubisco small subunit mature protein and three amino acids from the intervening cloning sequence. Rubisco, short for ribulose-

1,5-bisphosphate carboxylase/oxygenase, is the key enzyme in carbon assimilation. It is present in most autotrophic organisms including bacteria, algae and higher plants.[8]

Molecular analysis

The applicant confirmed by Southern blot analyses that a single copy of T-DNA I is integrated at a single integration locus in the genome of MON87708. Additionally, Southern blot analyses showed that backbone sequences of plasmid PV-GMHT4355 and the T-DNA II region containing the *cp4 epsps* cassette are absent in MON87708.

Sequence analysis of the MON 87708 insertion site and its flanking sequences demonstrated that the flanking regions consist of soybean DNA with one deletion and two insertions. During transformation, 899 bp of soybean genomic DNA were deleted from the insertion site. At the 5' end of the insert a 128 bp sequence was inserted during transformation. At the 3' end of the insert a 35 bp sequence was inserted during transformation. The inserted sequences do not appear to originate from plasmid PV-GMHT4355, *Agrobacterium* or soybean DNA, and have no apparent other source. According to the applicant, these molecular rearrangements probably resulted from double stranded break repair mechanisms in the plant during the *Agrobacterium* mediated tranformation process. The inserted sequences have been included in the bioinformatics analyses described below. The applicant states that there is no known function associated with the 899 bp deleted region.

The DNA sequences spanning the 5' and 3' junctions of the MON87708 insertion site and the genomic DNA, including the junctions with the inserted sequences during transformation, were bioinformatically analyzed from stop codon to stop codon for theoretical new fusion proteins (AD, TOX, and PRT databases, 2011). Results of these analyses demonstrated no structurally-relevant similarities between any known toxins or allergens and the putative polypeptides.

In conclusion, COGEM is of the opinion that the molecular characterization of MON87708 meets the criteria laid down by COGEM.[9]

Environmental risk assessment

The current application of soybean line MON87708 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. 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. Field trials with MON87708 soybean focusing on agronomic and phenotypic characteristics did not give any indication of increased weediness due to the presence of the DMO protein or the longer DMO protein. In view of the above, there are no reasons to assume that the introduced trait increases the ability of soybean seed to survive in the Dutch climatic conditions or increases the potential of MON87708 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 advise 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 (GS) 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 GS plan in this application 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 MON87708 soybean. In 2010, 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 GS plan sufficient for import and processing of MON87708 soybean.

Advice

COGEM has been asked to advise on import and processing for use in food and feed of dicamba tolerant soybean line MON87708. The molecular analysis of MON87708 is adequately performed.

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. There is no reason to assume that expression of the introduced *dmo* gene, resulting in production of a DMO protein and an alternatively processed DMO protein, will increase 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 the risk of spread of soybean MON87708 within the Netherlands due to incidental spillage of this soybean is negligible. Closely related species of soybean are not present in Europe and therefore introgression of the inserted gene into closely related species can not occur.

In COGEM's view, neither the expression of the DMO protein nor of the alternatively processed DMO protein starting with a pea Rubisco sequence gives a reason to assume that MON87708 will pose a risk to the environment.

Although the general surveillance (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 soybean line MON87708.

Based on the aspects discussed, COGEM is of the opinion that import and processing of soybean MON87708 poses a negligible risk to the environment. A food/feed safety assessment is carried out by other organisations. Therefore, COGEM abstains from advice on the potential risks of incidental consumption.

Literature

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