Import of genetically modified soybean DAS-81419-2 with two insect resistance traits

COGEM advice CGM/140506-01

Summary

The present application (EFSA/GMO/NL/2013/116) concerns import and processing for use in feed and food of the genetically modified soybean line DAS-81419-2. Cultivation is not part of this application.

Soybean DAS-81419-2 was generated by Rhizobium *mediated transformation of conventional soybean and expresses the* cry1F*v3*, cry1Ac*(synpro) and* pat *genes. As a result, soybean DAS-81419-2 is resistant against certain lepidopteran insects and tolerant to glufosinate-ammonium containing herbicides.*

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 characterization of DAS-81419-2 soybean meets the criteria of COGEM. COGEM considers the general surveillance plan adequate for import of DAS-81419-2, although some aspects can be improved.

In conclusion, COGEM is of the opinion that import and processing of soybean line DAS-81419-2 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 present application by Dow AgroSciences LLC (EFSA/GMO/NL/2013/116) concerns import, food, feed and processing of genetically modified (GM) soybean DAS-81419-2. This soybean was produced by *Rhizobium radiobacter* (formerly known as *Agrobacterium tumefaciens*[1]) mediated transformation and expresses two synthetic versions of *cry1* genes derived from *Bacillus thuringiensis*, and the *pat* gene isolated from *Streptomyces viridochromogenes*. As a result, soybean DAS-81419-2 is resistant against certain insect pests and tolerant to glufosinate-ammonium containing herbicides.

Previous COGEM advice

In the past COGEM advised positively on the import and processing of several *pat* containing soybean lines such as DAS-81419-2, DAS-68416-4, A2704-12 and A5547-127.[2,3,4,5,6] In 2010, COGEM advised positively on the import and processing of insect resistant soybean lines.[7,8] The lines express a Cry1Ac protein, however, the encoding gene is different from the synthetic *cry1Ac*(synpro) gene in DAS-81419-2.

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. 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.[9,10]

In the Netherlands, frost is common. On average 58 days in a year have a minimum temperature below 0°C.[11,12] In summer days are long, whereas soybean is a quantitative short-day plant. 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.[13,14,15] Further improvement of these varieties may eventually result in soybean varieties suited for commercial cultivation in the Netherlands. Due to the characteristics of soybean, COGEM is of the opinion that this development does not affect the environmental risk assessment of DAS-81419-2.

The soybean plant is not weedy in character.[9] Like for all domesticated crops, soybean has been selected for minimal seed scattering to reduce yield losses during harvesting. Soybean seeds rarely display dormancy and poorly survive in soil.[16] Soybean volunteers are rare throughout the world and do not effectively compete with other cultivated plants or primary colonisers.[9] In addition, volunteers are easily controlled mechanically or chemically.[9] COGEM is not aware of any reports of feral soybean populations in Europe.

Soybean is predominantly a self-pollinating species. The cross-pollination rate of soybean is an average of 1 to 3%.[9,17,18,19,20] The dispersal of pollen is limited because the anthers mature in the bud and directly pollinate the stigma of the same flower. In Europe, hybridisation with other species is not possible because there are no wild relatives of soybean.[9]

Molecular characterization

Soybean line DAS-81419-2 was generated by transforming conventional soybean with the disarmed *Rhizobium* strain EHA101. The *Rhizobium* strain was derived from strain A281 by inactivation of the T-DNA *onc* genes, and carried the helper plasmid pTiBo542 and the binary vector pDAB9582. The transformation was followed by conventional breeding, using glufosinate-ammonium as a selectable marker.

An overview of the T-DNA introduced in DAS-81419-2 (12,496 basepairs (bp)) is given below:

- 5’ Flanking border (soybean genomic DNA flanking the 5' end of the insert in DAS-81419-2 soybean)
- Re-arranged sequence at the 5’ end of the insert
- Complementary *cry1Ac*(synpro) partial fragment at the 5’end of the insert, 99% identical to the full-length *cry1Ac*(synpro) gene
- Re-arranged sequence at the 5’ end of the insert
- Last nucleotide from T-DNA Border B

- Intervening sequence
- RB7 MAR (matrix attachment region) partial sequence from the 7-5A gene of *Nicotiana tabaccum*
- Intervening sequence
- AtUbi10 promoter and the 5' untranslated region and intron from the *Arabidopsis thaliana* polyubiquitin 10 (UBQ10) gene
- Intervening sequence
- *Cry1F*v3 gene (synthetic version of the *cry1F* gene from *B. thuringiensis* subsp. *aizawa* strain PS811)
- Intervening sequence
- AtuORF23 3'UTR (3' untranslated region (UTR) sequence comprising the transcriptional terminator and polyadenylation site of open reading frame 23 (ORF 23) of *R. radiobacter* pTi15955)
- Intervening sequence
- CsVMV promoter and 5' UTR from *Cassava vein mosaic virus*
- Intervening sequence
- *Cry1Ac*(synpro) gene (synthetic version of the *cry1Ac* gene from *B. thuringiensis* subsp. *kurstaki* strain HD73)
- Intervening sequence
- AtuORF23 3'UTR (3' untranslated region (UTR) sequence comprising the transcriptional terminator and polyadenylation site of ORF23 of *R. radiobacter* pTi15955)
- Intervening sequence
- CsVMV promoter and 5' UTR from *Cassava vein mosaic virus*
- Intervening sequence
- *pat* gene (synthetic version of the phosphininothricin N-acetyl transferase (*pat*) gene derived from *S. viridochromogenes* providing tolerance to glufosinate ammonium)
- Intervening sequence
- AtuORF1 3'UTR (3' untranslated region (UTR) sequence comprising the transcriptional terminator and polyadenylation site of ORF1 of *R. radiobacter* pTi15955)
- Intervening sequence
- Rearranged DNA fragment at the 3' end of the insert
- 3' Flanking border (soybean genomic DNA flanking the 3' end of the insert in DAS-81419-2 soybean)

Southern blot analyses show that soybean DAS-81419-2 contains the T-DNA insert and a small T-DNA fragment, and that no plasmid backbone sequences are present in the soybean genome. Southern blot analyses also show that the insert is stably inserted and inherited in a Mendelian manner.

The applicant determined and analysed the sequences of the inserted sequences (12,496 bp), the 5' flanking region (1,297 bp), the 3' flanking region (1,379 bp), and the soybean genome insertion site. A comparison of the obtained sequences shows that the sequence of the inserted T-DNA

region is identical to the sequence of the T-DNA region of plasmid pDAB9582. In addition, a 9 bp rearranged sequence was integrated at the 3' end of the insert and a 135 bp rearranged sequence (containing a sub-fragment from *cry1Ac*(synpro)) was integrated at the 5' end of the insert. At the insertion site, there is a 57 bp deletion.

Analysis of the 5' and 3' flanking regions using the GenBank nucleotide and protein databases as well as the soybean scaffold sequence collection, indicates that the T-DNA fragments in soybean DAS-81419-2 are flanked by genomic sequences.

The applicant performed several bioinformatic analyses (BLASTn and BLASTx) on the region that was deleted during the integration of the insert in the soybean genome. No known gene or regulatory element was identified in the parental locus.

The applicant screened the junctions between elements within the T-DNA insert and the junctions between the insert and its borders for the presence of open reading frames (ORFs) from stop to stop codon. A total of 746 ORFs was identified. All the putative products of these ORFs were evaluated for potential similarities to known allergens and toxins. A sequence of 8 contiguous amino acids was identified matching with two high molecular weight glutenin allergens. According to the applicant, the identified sequence similarity does not relate to allergenic epitopes.

In summary, DAS-81419-2 soybean contains a single intact T-DNA insert in its nuclear genome. A 135 bp rearranged sequence (containing a sub-fragment from *cry1Ac*(synpro)) is integrated at the 5' end and a 9 bp rearranged sequence is integrated at the 3' end of the insert. At the insertion site 57 bp are deleted. No backbone sequences are present in the plant genome.

Bioinformatic analysis of ORFs spanning the junctions between the insert and the genome, and within the insert, identified no significant amino acid sequence similarities to known allergens or toxins harmful to humans or animals.

The molecular characterisation of soybean DAS-81419-2 meets the criteria laid down by COGEM.[21]

Properties of the introduced genes conferring insect resistance

Soybean line DAS-81419-2 contains the *cry1F*v3 and *cry1Ac*(synpro) genes. These *cry* genes encode delta-endotoxins (δ-endotoxin), which target certain lepidopteran insects. 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.[22,23]

Properties of the introduced gene conferring herbicide tolerance

DAS-81419-2 expresses the phosphinothricin acetyltransferase (PAT) protein. PAT confers tolerance to glufosinate-ammonium containing herbicides.[24] The active ingredient in glufosinate-ammonium is L-phosphinothricin (L-PPT), which binds to glutamine synthetase in plants. The

detoxification of ammonia is thereby prevented, leading to plant death. The PAT enzyme catalyses the conversion of L-PPT to an inactive form, which does not bind glutamine synthetase.[25]

The applicant states that the glufosinate-ammonium tolerance is only of importance during the selection stages of the genetic modification process. The trait has not been introduced for agronomic purposes.

Environmental risk assessment

The current notification concerns import and processing of soybean line DAS-81419-2. In case of spillage, DAS-81419-2 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.[9] In addition, volunteers are easily controlled mechanically or chemically.[9] There are no indications that soybean line DAS-81419-2 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 DAS-81419-2 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.[26] 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 authorization 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 authorization holder.

In 2010, COGEM published a report on the principles that, according to COGEM, should be followed for general surveillance.[27] COGEM considers the submitted GS plan adequate for import of DAS-81419-2 however, the plan could be improved on the following points.

In the present GS plan, the authorization holder states that the operators have agreed to provide information relevant to the monitoring of DAS-81419-2 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.[28] COGEM agrees with this request and points out that the applicant includes a statement on this point in the GS plan.[29]

Considerations and advice

COGEM has been asked to advise on import and processing for use in food and feed of soybean line DAS-81419-2. This GM soybean line expresses the synthetic *cry1F*v3 and *cry1Ac*(synpro) genes conferring resistance to certain lepidopteran insect pests. Additionally, the line expresses a *pat* gene providing tolerance to glufosinate- ammonium containing herbicides.

Although field trials have indicated that it might be possible to develop soybean varieties for cultivation in the Netherlands, 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 *cry1F*v3, *cry1Ac*(synpro) and *pat* genes will increase the potential of soybean to establish feral populations.

COGEM is of the opinion that the risk of spread of soybean DAS-81419-2 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 characterization of DAS-81419-2 soybean meets the criteria of COGEM. Although the GS plan could be improved, COGEM considers the submitted GS plan adequate for import of DAS-81419-2.

Based on the aspects discussed, COGEM is of the opinion that import and processing of soybean DAS-81419-2 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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