Import and processing of soybean line BPS-CV127-9

COGEM advice CGM/080921-01

Summary

The present notification by BASF Plant Science GmbH concerns the import and processing for use in feed and food of soybean line BPS-CV127-9 (hereafter referred to as CV127). Cultivation is not part of this application.

Due to the presence of a csr1-2 gene encoding an altered AtAHASL protein, soybean line CV127 is tolerant to imidazolinone containing herbicides. In the opinion of COGEM, the molecular analysis of soybean line CV127 is adequately performed.

In Europe, wild relatives of soybean are not present and soybean does not possess any of the attributes commonly associated with problematic weeds. In addition, survival of soybean is not possible in the Netherlands. Establishment of feral soybean populations has never been observed in European countries. There is no reason to assume that the inserted gene would introduce or increase the potential for soybean to establish feral populations. In Europe, hybridization with other species is not possible because there are no wild relatives of soybean present. Therefore, COGEM is of the opinion that incidental spillage of the soybeans will not pose a risk to humans and the environment.

In conclusion, COGEM is of the opinion that the risks to humans and the environment associated with import and processing of soybean line CV127 are negligible. COGEM points out that a food/feed safety assessment is carried out by other organizations. Therefore, COGEM abstained from advice on the potential risks of incidental consumption.

Introduction

The present application by BASF Plant Science GmbH, file EFSA/GMO/NL/2009/64, concerns the import and processing of soybean CV127 for use in feed and food. This line expresses the *csr1-2* gene from *Arabidopsis thaliana* conferring tolerance to imidazolinone containing herbicides.

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 meteorological 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, 16.8°C was the average summer temperature from 1971 to 2008. The average temperature of the three warmest summers since 1901 was 18.6°C (1). 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 (1). Moreover, during the Dutch growth season the days are long, whereas soybean is a quantitative short-day plant that needs short days for fructification.

Soybean is predominantly a self-pollinating species. The cross-pollination rate of soybean is less than 1% (2). 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 (3). Therefore, insect-born exportation of pollen is limited (2). In Europe, hybridization with other species is not possible because there are no wild relatives of soybean.

The soybean plant is not weedy in character (3). Cultivated soybean rarely displays dormancy (3) and seeds of cultivated soybean survive poorly in soil (4). Soybean volunteers are rare and do not effectively compete with other cultivated plants or primary colonizers (3). In addition, volunteers are easily controlled mechanically or chemically (3). Establishment of feral soybean populations has never been observed in European countries.

Molecular characterization

Soybean line CV127 was genetically modified by particle bombardment with a purified linear DNA fragment derived from plasmid pAC321 containing the following genetic elements:

- Arabidopsis gDNA; genomic DNA *Arabidopsis thaliana*
- At3g48570 5'UTR; 5' untranslated putative SEC61 gamma chain
- At3g48570 CDS; Putative SEC61 gamma chain coding sequence
- At3g48570 intron 1; SEC61 gamma chain intron 1 (interrupts coding sequence)
- At3g48570 3' UTR; 3' untranslated putative SEC61 gamma chain
- At3g48570 intron 2; SEC61 gamma chain intron 2
- At AHASL 5' UTR; Putative promoter and 5' untranslated region *aceto hydroxyacid synthase large subunit*
- Csr1-2 CDS; Coding sequence *aceto hydroxyacid synthase large subunit* with (S653N) point mutation
- At AHASL 3' UTR; 3'untranslated region *acetohydroxyacid synthase large subunit*
- Arabidopsis gDNA; genomic DNA *Arabidopsis thaliana*

Properties of the introduced genes conferring herbicide tolerance

Soybean line CV127 is tolerant to herbicides that contain imidazolinone. Soybean line CV127 expresses the acetohydroxyacid synthase large subunit (AHASL) gene also known as the *csr1-2* gene, which encodes an altered AtAHASL protein from *Arabidopsis thaliana* (5).

The AHASL enzyme is ubiquitous in plants and microbes and catalyzes the first step in the biosynthesis of the branched chain amino acids valine, leucine and isoleucine (6). In non-transgenic plants, inhibition of the AHAS enzyme by imidazolinone containing herbicides, leads to a deficiency in branched chain amino acids and other compounds derived from this pathway

that are needed for plant growth and development. Therefore, the application of imidazolinone herbicides leads to plant death in non-transgenic plants.

The AtAHASL protein encoded by *csr1-2* differs from the native AtAHASL protein by one amino acid substitution of a serine with an asparagine at residue 653 (S653N) which results in tolerance to imidazolinone containing herbicides. Besides the altered herbicide binding, the protein retains its biological function in the plant (7).

Molecular analysis

For the production of soybean line CV127, a DNA fragment was used that consisted mainly of genomic sequences from Arabidopsis, including the *csr1-2 gene*, the SEC61 gamma-chain gene, and unannotated DNA. The inserted DNA contains in addition to these sequences a partial copy (376bp) of the *csr1-2 gene at the 3' end of the insert*. A list of the inserted DNA components of soybean line CV127 is shown in the table below.

Genetic element	Size (bp)	Origin	Function
Insert			
Arabidopsis gDNA, unannotated	1051	*Arabidopsis thaliana*	no genes currently annotated in this region
At3g48570 5'UTR	61	*Arabidopsis thaliana*	5' untranslated putative SEC61 gamma chain
At3g48570 CDS	93 and 115	*Arabidopsis thaliana*	Putative SEC61 gamma chain coding sequence
At3g48570 intron 1	98	*Arabidopsis thaliana*	SEC61 gamma chain intron 1 (interrupts coding sequence)
At3g48570 3' UTR	19 and 203	*Arabidopsis thaliana*	3' untranslated putative SEC61 gamma chain
At3g48570 intron 2	472	*Arabidopsis thaliana*	SEC61 gamma chain intron 2
At *AHASL* 5' UTR and putative promoter	363	*Arabidopsis thaliana*	Putative promoter and 5' untranslated region for aceto hydroxyacid synthase large subunit
Csr1-2 CDS	2012	*Arabidopsis thaliana*	Coding sequence for aceto hydroxyacid synthase large subunit with (S653N) point mutation
At *AHASL* 3' UTR	217	*Arabidopsis thaliana*	3' untranslated region for aceto hydroxyacid synthase large subunit
Arabidopsis gDNA including a csr1-2 gene fragment	1002 376	*Arabidopsis thaliana*	Unannotated Arabidopsis DNA with an inserted partial copy (376bp) of the csr1-2 gene with a point mutation

The complete sequence of the DNA inserted in the CV127 genome was determined by sequencing PCR fragments. The applicant showed by Southern blot, PCR and sequence analysis

that besides a single functional copy of the *csr1-2* gene cassette, a partial *csr1-2* gene fragment and part of the SEC61γ subunit gene are integrated in the nuclear genome of soybean CV127. No vector sequences derived from the plasmid pAC321 are present. During the insertion into the soybean genome, 1275 bp of unannotated Arabidopsis genomic DNA at the 5'end and 500 bp at the 3'end of the insertion cassette were deleted. Furthermore, DNA sequence analysis revealed that the csr1-2 cassette contains three point mutations compared to the plasmid pAC321 from which the DNA fragment was derived to produce soybean line CV127. Two mutations are located downstream of the AtAHASL 3'untranslated region and are therefore genetically silent. The third point mutation is a G to A transition in the AHASL coding sequence. According to the applicant, this is a conservative amino acid substitution and has no impact on the herbicide tolerance or enzymatic properties of the AtAHASL protein.

Furthermore, the insert contains a duplicated fragment (376bp) of the csr1-2 coding sequence at the 3' integration point. This csr1-2 gene fragment also contains a point mutation. The insertion of this csr1-2 fragment created an ORF of 501bp that extends from the transgene insert into the 3'flanking sequence. Potential transcription of this ORF was investigated by RT-PCR, which indicated that no stable RNA is produced from this region and that the potential protein encoded by this ORF is not made. Furthermore, the inserted sequence contains the majority of the SEC61γ subunit gene, which is a component of the DNA fragment used for transformation. Based on RT-PCR results, the applicant states that the SEC61γ subunit gene is weakly transcribed in CV127 leaf tissue. COGEM is of the opinion that the weak expression of this protein, which plays a role in transport across the endoplasmatic reticulum, does not lead to an increased risk for humans or the environment since the SEC61γ protein is being expressed in most eukaryotic cells (8).

The 5' and 3' regions that flank the insert were amplified by PCR and sequenced. The obtained sequences were compared to soybean genomic DNA. This showed that the flanking sequences are of soybean origin. The presence of newly created open reading frames (ORF's) in the junctions between the transgene insert and the soybean genome were analyzed. The flanking regions were sequenced and analyzed for ORFs of one or more amino acids from stop codon to stop codon. The deduced amino acid sequences were analyzed. In total 24 ORF's were identified in the 5' junction and 6 ORF's in the 3' junction. None of the putative junction polypeptides showed significant homology with known toxins or allergens.

Recently, COGEM abstains from advices on the potential risks of incidental consumption in case a food/feed assessment is already carried out by other organizations. 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 systems which include the authorization holder and operators involved in the handling and use of viable CV127 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 CV127 soybean.

Advice

COGEM has been asked to advice on import and processing for use in food and feed of soybean line CV127. Because cultivation is not part of the present application, the risk assessment focuses on the accidental spillage of soybean.

COGEM points out that 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, wild relatives of soybean are not present in Europe and therefore introgression of the inserted genes into wild relatives cannot occur.

The molecular analysis of CV127 does not indicate that import and processing of this line would pose a risk to humans or the environment. In view of the risk assessment that was carried out by COGEM, COGEM considers the risks to man and the environment associated with import and processing of soybean CV127 negligible. COGEM points out that a food/feed assessment is carried out by other organizations. Therefore, COGEM abstained from advice on the potential risks of incidental consumption

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