Import and processing of genetically modified maize MON87411

COGEM advice CGM/151029-01

Summary

The present application (EFSA/GMO/NL/2015/124) concerns import and processing for use in feed or food of genetically modified maize MON87411. Cultivation is not part of this application.

Maize MON87411 was generated by Agrobacterium *mediated transformation of conventional maize and expresses the* DvSnf7 *suppression cassette and the* cry3Bb1 *and* cp4 epsps *genes. As a result, maize MON87411 is resistant to coleopteran insects such as Western corn rootworm* (Diabrotica *spp.) and tolerant to glyphosate containing herbicides.*

During its long domestication process, maize has lost its ability to survive in the wild. In the Netherlands, the appearance of maize volunteers is rare and establishment of volunteers in the wild has never been reported. There are no reasons to assume that the introduced traits will allow maize MON87411 to establish feral populations. The introduced sequences cannot introgress into other species since wild relatives of maize are not present in Europe. COGEM is of the opinion that incidental spillage of MON87411 poses a negligible risk to the environment.

The molecular characterisation of MON87411 maize meets the criteria of COGEM.

In view of the above, COGEM considers the environmental risks associated with import and processing of maize MON87411 to be negligible. COGEM abstains from giving advice on the potential risks of incidental consumption because a food/feed assessment is already carried out by other organisations.

Introduction

The scope of the present notification by Monsanto Europe S.A. concerns import and processing of genetically modified maize MON87411 for use in feed and food. This maize is produced by *Agrobacterium tumefaciens* mediated transformation of maize line LH244 and expresses the *DvSnf7* suppression cassette, the *cry3Bb1* gene and the *cp4 epsps* gene. As a result, maize MON87411 is resistant to coleopteran insects such as Western corn rootworm (*Diabrotica virgifera virgifera*) and tolerant to glyphosate containing herbicides.

Previous COGEM advice

COGEM has not yet advised on import and processing of MON87411 maize. In 2007, COGEM advised positively on import and processing of maize line MON88017.[1] According to the applicant, the *cry3Bb1* and c*p4 epsps* genes expressed in this line are identical to the *cry3Bb1* and c*p4 epsps* genes expressed in MON87411.

Aspects of the crop

Maize (*Zea mays*) is a member of the grass family *Poaceae*. Maize is a highly domesticated crop, originating from Central America, but nowadays maize is cultivated globally. Maize is predominantly wind pollinated.[2,3] Insect pollination is limited since the female flowers do not

produce nectar and are therefore not attractive to insect pollinators.[4] In Europe, no wild relatives of maize are present and therefore hybridisation with other species cannot occur.

In the Netherlands, the appearance of volunteers is very rare to absent.[5] Domesticated maize requires warm conditions in order to grow and does not tolerate prolonged cold and frost.[4,6] The seeds (kernels) remain on the cob after ripening and do not shatter naturally.[4,7] In cultivation areas with warmer climatic conditions, the appearance of volunteers can occur the year following maize cultivation due to spilled cobs or kernels. However, these volunteers are usually killed by common mechanical pre-planting soil preparation practices.[4]

Maize is very sensitive to weed competition.[8] During the long process of domestication, maize has lost the ability to survive in the wild.[3] Establishment of feral maize populations has never been observed in the Netherlands and COGEM is not aware of any reports of wild maize plants elsewhere in Europe.

Molecular characterisation

Maize MON87411 was produced by *A. tumefaciens* mediated transformation of maize line LH244 with plasmid PV-ZMIR10871. The T-DNA contains the *DvSnf7b* suppression cassette, the *cry3Bb1* expression cassette and the *cp4 epsps* expression cassette. The plasmid backbone contains, amongst other things, the *aadA* antibiotic resistance gene which is used as a selection marker.

An overview of the T-DNA introduced in MON87411 is given below:

- T-DNA Left border region of *A. tumefaciens.*
- *E9.* 3' UTR of the *rbcS* gene family from *Pisum sativum*.
- DvSn7. Partial coding sequence of the *Snf7* gene.
- DvSn7. Partial coding sequence of the *Snf7* gene.
- *Hsp70.* Intron and flanking exon of the *hsp70* gene from maize.
- *e35S*. Promoter from the 35S RNA of *Cauliflower mosaic virus* (CaMV).
- *pIIG*. Promoter sequence of the *pIIG* gene from maize.
- *Cab*. 5'UTR leader sequence from the chlorophyll a/b binding protein of *Triticum aestivum.*
- *Ract1*. Intron and flanking UTR sequence of the *act1* gene from *Oryza sativa.*
- CS6-*cry3Bb1*. Codon optimized sequence from the *cry3Bb1* gene of *Bacillus thuringiensis*.
- *Hsp17*. 3'UTR sequence from heat shock protein Hsp17 of *T. aestivum.*
- *TubA.* Promoter, 5'UTR leader and intron sequences of the *OsTubA* gene family from *O. sativa.*
- *CTP2*. Targeting sequence of the *ShkG* gene from *Arabidopsis thaliana*.
- *cp4 epsps*. Coding sequence of the *cp4 epsps* gene from *Agrobacterium* sp. Strain CP4.
- *TubA.* 5'UTR leader and intron sequences of the *OsTubA* gene family from *O. sativa.*
- B-Right border region of *A. tumefaciens.*

Characterisation of the number of T-DNA insertions in MON87411 was conducted using a combination of Next Generation Sequencing (NGS) and Junction Sequence Analysis (JSA).[9,10] In addition, directed sequencing (locus specific PCR on non-fragmented genomic DNA and sequence analyses) was used to verify the sequences of the DNA insert and the adjacent flanking DNA.

The strategy of NGS is to produce DNA sequence fragments that comprehensively cover the entire genome of test and control plants. JSA is used to characterise new junctions between the introduced DNA and the native genomic DNA sequence.

For NGS, short (approximately 100 bp) randomly distributed sequenced fragments (sequencing reads) were generated and all reads were mapped to a known single copy endogenous gene (*Pyruvate decarboxylase, pdc3*) to determine the coverage. According to the applicant, the analysis showed that *pdc3* was covered by 100-mers at >107x for each sample.

To determine the presence of backbone and T-DNA sequences, the number of insertions and the T-DNA copy number, NGS/JSA was performed. Based on this analysis, the applicant concludes that MON87411 maize contains one copy of the T-DNA insert at a single integration locus and that no backbone sequences of plasmid PV-ZMIR10871 are present in the maize genome. The applicant further concludes that the insert is stably inserted.

By 'directed sequencing', the applicant determined the sequence of the insert and the flanking DNA. The applicant concludes that the inserted T-DNA is identical to the T-DNA of plasmid PV-ZMIR10871 with the exception of small terminal deletions at the Left and Right border regions. There was also a 118 bp deletion of genomic DNA at the insertion site. BLASTn and BLASTx analyses were performed to determine if the insertion of the T-DNA had disrupted any endogenous ORFs located in the maize genomic DNA flanking the insert. According to the applicant, these analyses indicated that it is unlikely that endogenous ORFs at the insertion site were disrupted.

The applicant analysed the junctions between the insert and the maize genome sequence from stop codon to stop codon for the presence of novel open reading frames (ORFs). A total of 8 ORFs encoding putative proteins of 8 aminoacids or greater were identified and evaluated for potential sequence similarities to known allergens, toxins and biologically active proteins. The applicant found no similarities.

The applicant states that bioinformatical analysis on the putative peptides encoded by translation of reading frames 1 through 6 of the T-DNA-I insert did not demonstrate similarities to known allergens, toxins and biologically active proteins, apart from the introduced *cry3Bb1* and *cp4 epsps* genes.

In summary, MON87411 maize contains a single intact T-DNA insert. At the insertion site 118 bp are deleted. No backbone sequences are present in the plant genome. Bioinformatic analysis of ORFs spanning the insert-genome junctions and within the insert identified no significant amino

acid sequence similarities to known allergens, toxins and biologically active proteins. The molecular characterisation of maize MON87411 meets the criteria laid down by COGEM.[11]

Properties of the introduced sequences

cry3Bb1

MON87411 contains the *cry3Bb1* gene encoding the Cry3Bb1 protein. When this protein is ingested by susceptible insects (e.g. Western corn rootworm), it is proteolytically cleaved in the midgut of the insect. The resulting delta-endotoxin binds to specific receptors on the epithelial surface of the midgut of susceptible insects, which causes the formation of pores. This leads to disruption of the movement of solutes across the gut epithelium and ultimately in death of the insect.[12,13]

DvSnf7

The *DvSnf7b* suppression cassette expresses an inverted repeat sequence designed to match the *Snf7* sequence in Western corn rootworm. Expression of the suppression cassette results in the formation of a dsRNA transcript containing a 240bp fragment of the *Snf7* gene. Via the RNA interference (RNAi) pathway, the dsRNA mediates suppression of the endogenous Snf7 protein in the Western corn rootworm. Snf7 is part of the Endosomal Sorting Complex Required for Transport III (ESCRT-III) complex.[14] This complex is involved in the sorting of transmembrane proteins en route to lysosomal degradation via the endosomal-autophagic pathway.[15] Suppression of Snf7 leads to accumulation of ubiquitinated proteins destined for lysosomal degradation.[14] This accumulation impairs homeostasis and results in cellular death and Western corn rootworm mortality.[14,16]

Properties of the introduced gene conferring herbicide tolerance

Maize line MON87411 also expresses the 5-enolpyruvylshikimate-3-phosphate synthase (*cp4 epsps*) gene. The *cp4 epsps* gene encodes the CP4 EPSPS protein. EPSPS is an 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. In contrast to EPSPS, the CP4 EPSPS protein is not inhibited by glyphosate and therefore MON87411 is tolerant to glyphosate containing herbicides.[17]

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.[18] 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/feed assessment for Regulation (EC) 1829/2003 applications is carried out by RIKILT. The outcome of the assessment by these organisations (EFSA, RIKILT) was not known upon completion of this advice.

Considerations and advice

COGEM has been asked to advice on import and processing of genetically modified maize line MON87411. Maize MON87411 was generated by *Agrobacterium* mediated transformation of conventional maize and expresses the DvSnf7 suppression cassette and the *cry3Bb1* and *cp4 epsps* genes. As a result, maize MON87411 is resistant to coleopteran insects such as Western corn rootworm (*Diabrotica* spp.) and tolerant to glyphosate containing herbicides.

Maize has lost the ability to survive in the wild. In the Netherlands, volunteers are rare and establishment of maize plants in the wild has never been observed. COGEM is of the opinion that the risk of spread of MON87411 maize within the Netherlands due to incidental spillage of MON87411 is negligible. There is no reason to assume that the introduced traits increase the potential of maize MON87411 to establish feral populations. In addition, introgression of the introduced sequences into closely related species cannot occur, as wild relatives of maize are not present in Europe.

The molecular characterisation of MON87411 maize meets the criteria of COGEM.

COGEM has published several recommendations for further improvement of the general surveillance (GS) plan[19,20] but considers the current GS plan adequate for import and processing of MON87411.

In view of the above, COGEM is of the opinion that import and processing of maize line MON87411 poses a negligible risk to the environment.

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