Import and processing of maize line MON 87427 with tissue-selective tolerance to glyphosate

COGEM Advisory report CGM/130314-01

Summary

The present application (file EFSA/GMO/BE/2012/110) concerns import and processing for use in feed and food of the genetically modified maize line MON 87427. Cultivation is not part of this application.

Maize line MON 87427 selectively expresses the cp4 epsps *gene in vegetative and female reproductive tissues. As a result, the maize line is tolerant to glyphosate containing herbicides with the exception of the male reproductive tissues.*

The applicant showed by Southern blot analyses that MON 87427 contains one copy of the insert at a single integration locus and that the backbone of the plasmid used for transformation is absent. Bioinformatic analysis of the junctions of the inserted sequences and the maize genomic DNA, identified fourteen open reading frames (ORFs). The translated sequences from these ORFs were analysed for similarity to known toxins or allergens. No similarities were found. The molecular characterization of maize line MON 87427 meets the criteria of COGEM.

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 trait will increase the potential of maize to establish feral populations. The introduced gene cannot spread to closely related species since wild relatives of maize are not present in Europe. COGEM considers the appended General Surveillance plan adequate for import of MON 87427.

In view of the above, COGEM is of the opinion that incidental spillage of MON 87427 poses a negligible risk to the environment. COGEM abstains from giving advice on the potential risks of incidental consumption since a food/feed assessment is already carried out by other organizations. COGEM considers the environmental risks associated with import and processing of maize line MON 87427 to be negligible.

Introduction

The scope of the present application (EFSA/GMO/BE/2012/110) filed by Monsanto Europe S.A. concerns import, food, feed and processing of maize line MON 87427. This maize line was produced by *Rhizobium radiobacter* (previously known as *Agrobacterium tumefaciens*[1]) mediated transformation of conventional variety LH198 x Hill and expresses the *cp4 epsps* gene from *Agrobacterium* sp. strain CP4 in vegetative and female reproductive tissues. As a result, the maize line is tolerant to glyphosate containing herbicides with the exception of the male reproductive tissues.

Previous COGEM advice

MON 87427 has not been previously assessed by COGEM. However, in the past COGEM has advised positively on the import and processing of several *cp4 epsps* containing maize events such as NK603 and MON88017 and several stacked events of these lines.[2,3]

Aspects of the crop

Maize (*Zea mays* L.) is a member of the grass family *Poaceae*. Maize is a highly domesticated crop, originating from Central America. Domesticated maize probably arose about 9000 years ago from a single domestication event in Mexico as a result of human selection of the annual teosinte *Z. mays* subsp. *parviglumis*.[4]

Maize is predominantly wind pollinated.[5,6] Although pollination by bees and other insects cannot be completely excluded, insect pollination is limited since the female flowers do not produce nectar and are therefore not attractive to insect pollinators. [7] 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.[8] Domesticated maize requires warm conditions in order to grow and does not tolerate prolonged cold and frost.[7,9] The seeds (kernels) remain on the cob after ripening and do not shatter naturally.[7,10] 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.[7] Maize is very sensitive to weed competition.[11]

During the long process of domestication, maize has lost the ability to survive in the wild.[6] Establishment of maize plants in the wild has never been observed in the Netherlands and COGEM is not aware of any reports of wild maize plants elsewhere in Europe.

Molecular characterisation

MON 87427 was produced by *R. radiobacter* mediated transformation of hypocotyls from the conventional maize line LH198xHill using vector PV-ZMAP1043. The following elements were introduced in MON 87427:

- Left border region; DNA region from *R. radiobacter* containing the Left Border sequence used for transfer of the T-DNA;
- P- *e35S*; Promoter for the *Cauliflower mosaic virus* (CaMV) 35S RNA containing the duplicated enhancer region that directs transcription in plant cells;
- I- *hsp70*; First intron from the maize heat shock protein 70 gene;
- TS- CTP2; Targeting sequence from the *ShkG* gene encoding the chloroplast transit peptide region of *Arabidopsis thaliana* EPSPS that directs transport of the CP4 EPSPS protein to the chloroplast;
- CS- *cp4epsps;* Codon-optimized coding sequence of the *aroA* gene from the *Agrobacterium* sp. strain CP4 encoding the CP4 EPSPS protein;

- T- *nos*; 3' non-translated region of the nopaline synthase (*nos*) gene from *Rhizobium radiobacter* which terminates transcription and directs polyadenylation;
- B-Right border region; DNA region from *R. radiobacter* containing the Right Border sequence used for transfer of the T-DNA.

According to the applicant, the *e35S-hsp70* promoter and intron combination is used to drive CP4 EPSPS protein expression in vegetative and female reproductive tissues, conferring tolerance to glyphosate in the leaves, stalk and root tissues and tissues that develop into seed and silks. This specific promoter and intron combination results in limited or no production of CP4 EPSPS protein in two key male reproductive tissues: pollen microspores, which develop into pollen grains, and tapetum cells that supply nutrients to the pollen. The applicant states that the absence of tolerance in male reproductive tissues allows glyphosate-treated MON 87427 containing inbred lines to serve as a female parent in the production of hybrid seed.

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 the plant is tolerant to glyphosate containing herbicides.[12]

Molecular analysis

The applicant used Southern blot analyses to determine the number of insertion sites and copies of the integrated T-DNA as well as the presence or absence of plasmid vector backbone sequences. The applicant demonstrated that maize MON 87427 contains one copy of the insert at a single integration locus and that it does not contain backbone sequences of plasmid PV-ZMAP1043.

The applicant determined the sequence of the insert and adjacent flanking genomic DNA sequences in MON 87427. A comparison with the sequence of the conventional control LH198 x HiII revealed a 140 bp deletion and 41 bp insertion at the 5' insert-to-flank junction and a 24 bp insertion at the 3' insert-to-flank junction.

The junctions between the T-DNA insert and the flanking plant genomic DNA were sequenced and screened for potential newly created open reading frames (ORFs). In total, fourteen ORFs were identified. The amino acid sequences of these ORFs were compared with known allergens and toxins utilizing the AD_2012, TOX_2012, and PRT_2012 databases. The analysis showed no biologically significant sequence similarities with known toxins, allergens or biologically active proteins.

Bioinformatic analyses were also performed to assess the potential of allergenicity, toxicity, or biological activity of the putative peptides encoded by the six reading frames translated from the T-DNA sequence present in MON 87427. The analysis also showed no biologically significant sequence similarities with known toxins, allergens or biologically active proteins.

In view of the above, COGEM is of the opinion that the molecular characterisation of MON 87427 has been adequately performed and meets the criteria laid down by COGEM.[13]

COGEM abstains from giving advice on the potential risks of incidental consumption since a food/feed assessment is already carried out by other organisations.[14] This application is submitted under Regulation (EC) 1829/2003, therefore a food/feed assessment is carried out by EFSA. Other organisations 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 organisations (EFSA, RIKILT) was not known at the moment of the completion of this advice.

General Surveillance

General surveillance (GS) has been introduced to be able to observe unexpected adverse effects of genetically modified (GM) crops on the environment. The setting or population in which these effects might occur is either not, or hardly predictable. The GS plan is required for every application for market authorisation. The current GS plan states that unanticipated adverse effects will be monitored by the authorisation holder and operators involved in the handling and use of viable maize MON 87427. The authorisation holder will also examine possibilities for the involvement of existing monitoring networks.

In 2010, COGEM published a report on the principles that, according to COGEM, should be followed for general surveillance.[15] COGEM considers the widely used GS plan, accompanying the current application, adequate for import of MON 87427. However, the GS plan could be improved by a guarantee that operators will monitor for unanticipated effects .

COGEM points out that adding a statement that the applicant will make raw data and analysis of monitoring data available to the Competent Authorities and the European Commission, could improve the GS plan.[16] This request is also made by EFSA in the guidance document.[17]

The GS plan 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.

Advice

COGEM has been asked to advice on import and processing of maize line MON 87427. This maize line selectively expresses the *cp4 epsps* gene conferring tolerance to glyphosate in the leaves, stalk and root tissues and tissues that develop into seed or grain and silks.

Maize has lost the ability to survive in the wild. In addition, maize needs human intervention to disseminate its seed. In the Netherlands, volunteers are rare and establishment of maize plants in the wild has never been observed. There is no reason to assume that expression of the *cp4 epsps* gene increases the potential of maize to establish feral populations in case of incidental spillage. Introgression of the introduced gene into closely related species cannot occur, as wild relatives of maize are not present in Europe.

COGEM considers the current GS plan sufficient for import and processing of maize line MON 87427. The molecular characterization of maize line MON 87427 meets the criteria of COGEM.

In view of the above, COGEM is of the opinion that the risks for humans and the environment associated with import and processing of maize line MON 87427 are negligible. A food/feed safety assessment is carried out by other organizations. Therefore, COGEM abstains from advice on the potential risks of incidental consumption.

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