Import and processing of genetically modified maize line 98140

COGEM advice CGM/090116-01

The present application by Pioneer Hi-Bred International Inc. (file EFSA/GMO/UK/2008/53*) concerns import and processing for use in feed and food of the genetically modified maize line 98140. Cultivation is not part of this application.*

Maize line 98140 has been genetically modified by insertion of the zm-hra and gat4621 genes resulting in tolerance to glyphosate containing and ALS inhibiting 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 increase the potential of maize to establish feral populations. The introduced genes cannot spread to closely related species since wild relatives of maize are not present in Europe. Therefore, COGEM is of the opinion that incidental spillage of maize line 98140 will probably not pose a risk to the environment.

However, COGEM points out that the molecular analysis of maize line 98140 does not meet the criteria of COGEM as the applicant restricted the analysis of putative open reading frames at the junction between maize genomic DNA and the T-DNA fragment to amino acid sequences that start with a methionine residue.

Although COGEM is of the opinion that import and processing of this maize line will not pose a risk to the environment, in view of the incomplete molecular characterization COGEM cannot advise positively on the application for import and processing of maize line 98140.

Introduction

The scope of the present notification (EFSA/GMO/UK/2008/53) by Pioneer Hi-Bred International Inc. concerns import and processing of maize line 98140. The genetically modified maize line 98140 expresses the genes *gat4621* and *zm-hra* which confer tolerance to glyphosate containing and ALS inhibiting herbicides.

Previous COGEM advices

In 2008, COGEM advised positively on a small scale (category 1) field trial with maize line 98140 (1). In addition, COGEM advised on import and processing of soybean 356043. This soybean line contains the *gat4601* and *gm-hra* genes which are similar to the *gat4621* and *zm-hra* genes that are present in maize line 98140 (2).

Aspects of the crop

Maize (*Zea mays L.*) is a member of the grass family *Poaceae.* Maize is cultivated as an agricultural crop, originating from Central America. Although insect pollination cannot be completely excluded, maize is predominantly wind pollinated (3, 4). According to literature, pollen viability varies between 30 minutes and 9 days (4, 5, 6). In Europe, no wild relatives of maize are present and, therefore, hybridization with other species cannot occur.

The appearance of volunteers is very rare under Dutch conditions. Grains exhibit no germination dormancy, resulting in a short persistence. In addition, only few seeds remain on the field after the harvest of fodder maize (3). Establishment of maize plants in the wild has never been observed in the Netherlands.

Molecular characterization

The genetically modified maize line 98140 which expresses the *gat4621* and *zm-hra* genes, was produced by *Agrobacterium tumefaciens*-mediated transformation. An overview of the introduced sequences is given below:

- right border region derived from the Ti-plasmid of *A. tumefaciens*
- *pin*II terminator from the proteinase inhibitor II gene of *Solanum tuberosum*
- *zm-hra* gene, an optimized form of the acetolactate synthase (*als*) gene of *Z. mays* containing two nucleotide changes compared to the endogenous *als* gene resulting in two point mutations in the protein sequence
- *als* promoter from the *als* gene of *Z. mays* providing constitutive expression
- CaMV 35S enhancer (three copies) from the *Cauliflower mosaic virus* which enhances expression of the introduced genes
- *ubi*ZM1 promoter from the ubiquitin gene of *Z. mays* providing constitutive expression
- *ubi*ZM1 5' untranslated region and intron from the ubiquitin gene of *Z. mays*
- *gat4621* gene, encoding glyphosate N-acetyltransferase. The *gat4621* gene was produced by multi-gene shuffling of three different *gat* alleles from *Bacillus licheniformis* and by introducing an additional alanine residue. Additional genetic diversity from *Bacillus cereus, Bacillus subtilis*, *Listeria inocua* and *Zymomonas mobilis* was introduced by PCR incorporation of oligonucleotides and subsequent gene shuffling
- *pin*II terminator region from the proteinase inhibitor II gene of *S. tuberosum*
- left border region derived from the Ti-plasmid of *A. tumefaciens*

Properties of the introduced genes conferring herbicide tolerance

Maize line 98140 expresses the *zm-hra* gene which confers tolerance to acetolactate synthase (ALS) inhibiting herbicides. ALS inhibiting herbicides bind to the ALS enzyme which is required for the production of branched chain amino acids (valine, leucine and isoleucine) (7). This results in the production of reduced quantities of branched chain amino acids and a shortage of these amino acids. This shortage leads to rapid inhibition of cell division and subsequently to plant death (7). The *zm-hra* gene encodes an ALS protein that is insensitive to ALS inhibiting herbicides thus conferring tolerance to ALS inhibiting herbicides.

In addition, maize line 98140 expresses the *gat4621* gene which leads to glyphosate resistance. Glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme involved in the biosynthesis of aromatic amino acids (8). Application of glyphosate results in a lack of amino acids essential for growth and development of plants. Maize line 98140 expresses the *gat4621* gene which produces a glyphosate N-acetyltransferase enzyme that detoxifies glyphosate by acetylating glyphosate into N-acetylglyphosate (9).

Molecular analysis

Southern blot hybridization was used to determine whether backbone sequences were present in maize line 98140. None of the five probes that were used hybridized to maize line 98140. However, the vector backbone is not fully covered by these five probes. In *A. tumefaciens*-mediated transformation backbone sequences are usually co-transferred with the T-DNA region. Therefore, if backbone sequences are present in transformants, these usually include the regions adjacent to the T-DNA region. Three of the probes used for Southern blot hybridization were located in the regions that flank the T-DNA fragment (two of them adjacent to the left and right border regions). Since these probes did not hybridize to maize line 98140, COGEM considers it unlikely that other parts of the vector backbone were transferred to maize line 98140.

The applicant demonstrated by Southern blot analysis that a single T-DNA fragment was inserted in maize line 98140. Southern blot and sequence analysis showed that the T-DNA fragment was intact. The DNA sequence of the T-DNA fragment in maize line 98140 was compared to the DNA sequence of the T-DNA region in the vector used for transformation. The comparison showed that thirty basepairs of the right border end and twenty-four basepairs of the left border end are not present in maize line 98140. The T-DNA fragment in maize line 98140 consists of 7386 basepairs.

Bioinformatic analysis of the regions that flank the T-DNA fragment shows that the flanking regions are homologous to maize genomic DNA. These results demonstrate that

the insert was sequenced completely. In addition, PCR analysis confirmed that the flanking regions were present in non-genetically modified maize lines.

The junctions between the maize genomic DNA and the T-DNA fragment were analyzed for the presence of putative open reading frames. The putative open reading frames were translated *in silico* into amino acid sequences. The applicant stated that as the presence of a methionine residue is needed for the start of protein translation, only amino acid sequences that start with such a residue were analyzed for homology with known proteins. No homology to known proteins was found. In its recent publication on the molecular characterization of commercial releases of genetically modified crops COGEM points out that protein translation can occur without the presence of an ATG start codon. In addition, as a result of splicing several smaller open reading frames may be coupled and translated into protein. In view of these considerations COGEM is of the opinion that putative open reading frames should be identified from stop to stop codon. At the junctions between the maize genomic DNA and the T-DNA fragment all identified putative open reading frames should be completely analyzed for homology to known proteins (10). As the applicant limited the analysis to amino acids that begin with a methionine residue the molecular characterization of maize line 98140 does not meet the criteria of COGEM.

General surveillance

General surveillance has been introduced to be able to observe unexpected effects of the cultivation of genetically modified crops on the environment. The setting or population in which these effects might occur is either not, or hardly predictable.

In the present application, a detailed general surveillance plan is provided to observe and register adverse effects of the import of maize line 98140 timely. Following the initial placing on the market, the authorization holder will submit general surveillance reports on an annual basis for the duration of the authorization period.

Observations for unanticipated adverse effects will be monitored by existing systems which include the authorization holder and operators involved in the handling and use of viable 98140 maize. Operators involved in the import, handling and processing of 98140 maize inform the European trade associations (COCERAL, UNISTOCK and FEDIOL) of observed adverse effects. The trade associations report these effects to the authorization holder via the European Association of Bioindustries (EuropaBio) or directly to the authorization holder. EuropaBio is an association of members of the plant biotechnology industry which hosts a website containing information on approved genetically modified plants subject to general surveillance. The website contains an e-mail address and a telephone number to exchange information on the plants. COGEM points out that to gather general surveillance data a questionnaire would be helpful. By placing such a list

on the website, essential information on adverse effects can be collected in a more coherent and consistent manner.

As mentioned in previous advices, COGEM prefers independent organizations which have expertise on the environment and whose activities in general surveillance continue after the authorization period, to be involved in general surveillance. In a previous advice on post-market monitoring, COGEM has outlined the standards that have to be met by a post-market monitoring system and has identified organizations which could be involved in post-market monitoring in the Netherlands (11).

Advice

COGEM has been asked to advice on import and processing of maize line 98140.

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. There is no reason to assume that the expression of the *zm-hra* and *gat4621* genes in maize line 98140 increases the potential of maize to establish feral populations. In addition, introgression of the introduced genes into closely related species cannot occur, as wild relatives of maize are not present in Europe. In view of the above, COGEM is of the opinion that incidental spillage of maize will probably not pose a risk to humans and the environment.

In COGEM's view it is sufficiently proven that a single intact T-DNA fragment is present in maize line 98140. The sequence of the entire T-DNA fragment was determined. In addition, putative open reading frames at the junctions between the T-DNA fragment and the maize genomic DNA were translated *in silico* into amino acid sequences. Only amino acid sequences that start with a methionine residue were analyzed for homology to known proteins. COGEM is of the opinion that all putative open reading frames (identified from stop to stop codon) at the junction regions should be completely analyzed for homology to known proteins. As the applicant limited the analysis to amino acids that begin with a methionine residue the molecular characterization of maize line 98140 does not meet the criteria of COGEM.

In conclusion, COGEM is of the opinion that import of maize line 98140 poses a negligible risk to the environment. However, as the molecular characterization contains flaws COGEM cannot advice positively on import and processing of maize line 98140.

References

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