Import of genetically modified maize LY038 x MON810

COGEM advice CGM/070504-02

This notification concerns the import and processing of the genetically modified maize line LY038xMON810 for use in food and feed. Cultivation is not part of the application. The maize line harbors the gene cordapA conferring a higher lysine content. Also, the cry1Ab gene is present, conferring resistance to the larvae of certain butterflies and moths (Lepidoptera). In 2006, in the Philippines, an application was granted for use in food en feed.

Recently, COGEM issued an advice on the parental maize line LY038. COGEM stated that there was a lack of information regarding the molecular analysis. In particular incomplete data were presented on the analysis of the flanking sequences. COGEM was of the opinion that the environmental risks of the import of LY038 probably are negligible. However, due to the lacking data concerning the molecular characterisation, this opinion could not be sufficiently substantiated. Therefore, in COGEMs view, the applicant has to provide the missing data before a final judgement can be issued.

Introduction

The scope of the present notification concerns the import and processing of maize line LY038xMON810 for use in feed and food. Cultivation is not included in the scope of this notification and is therefore not addressed in this advice. In 2006, the maize line is authorized for food and feed purposes in the Philippines (1).

The maize line is produced by traditional crossing of maize lines LY038 and MON810. Maize line LY038 expresses the gene *cordapA* conferring higher lysine content. Maize line MON810 expresses *cry1Ab* conferring resistance to certain lepidopteran insects such as the European corn borer.

Previous COGEM advices

Recently, COGEM advised on the import and processing of parental maize line LY038 which expresses the gene *cordapA* (2). COGEM stated that there was a lack of information regarding the molecular analysis. She was of the opinion that the environmental risks of the import of LY038 probably are negligible. However, in view of the lacking data concerning the molecular characterisation, this opinion could not be substantiated. Therefore, in COGEMs view the applicant had to provide the missing data.

In 1996, COGEM advised positively on the cultivation of the other parental maize line MON810, containing the gene *cry1Ab* (3). Additionally, maize line MON810 is already commercially grown world wide including Europe.

Aspects of the crop

Maize (*Zea mays L.*) is a member of the grass family *Poaceae.* Maize is being cultivated as an agricultural crop, originating from Central America. Although insect pollination can not be completely excluded, maize is predominantly wind pollinated (4-5). According to literature, pollen viability varies between 30 minutes and 9 days (5-7). In Europe, no wild relatives of maize are present and, therefore, hybridization with other species can not occur.

The appearance of volunteers is very rare under Dutch conditions. Grains exhibit no germination dormacy, resulting in a short persistence. In addition, only few seeds remain on the field after harvesting of fodder maize (4). Establishment of maize plants in the wild has never been observed in the Netherlands.

Molecular characterisation

The genetically modified maize line LY038 was produced by particle acceleration and by means of the *cre/lox* recombination system. A detailed description of the construction of the parental maize line LY038 can be found in another COGEM advice (2).

An overview of the sequences introduced in LY038 is given below:
cordapA expression cassette:

- Glb1 promoter, originating from *Z. mays*
- rAct1 intron, intron from the rice (*Oryza sativa*) actin gene: promotes transcription
- mDHDPS CTP, chloroplast targeting sequence from dihydrodipicolinate synthase (DHDPS) derived from *Z. mays*
- *cordapA*, coding sequence from the dihydrodipicolinate synthase (*dapA*) gene derived from *Corynebacterium glutamicum*
- Glb1 3'UTR, 3'nontranslated region from the *Globulin 1* (Glb1) gene from *Z. mays*: directs the polyadenylation of the mRNA

nptII gene cassette:

- *LoxP-2*, recombination site recognized by Cre recombinase originating from bacteriophage P1
- CaMV 35S promoter, originating from *Cauliflower mosaic virus*
- *nptII*, coding sequence for neomycin phophotrasferase type II, derived from Eschericia coli.

- *Ble*, 153 bp fragment of the 378 bp gene for *bleomycin* originating from *E.coli*
- NOS 3', non translated region of the nopaline syntase (NOS) gene from *Agrobacterium tumefaciens*
- *LoxP-1,* recombination site

Summary of the sequences inserted in MON810:
cry1Ab gene cassette:

- P-e35S, originating from the CaMV, containing a partial sequence of the CaMV promoter with a duplicated enhancer region and 5' untranslated region
- Zmhsp70 intron, DNA sequence derived from maize containing the intron sequence from the maize *hsp70* gene (heat shock protein) present to stabilize the level of gene transcription
- *cry1Ab*, DNA sequence containing synthetic linker and a portion of the synthetic coding sequence for a variant of the Cry1Ab protein from *B. thuringiensis subsp. Kurstaki.*

Properties of the introduced genes conferring increased lysine content
The transgenic maize line LY038 was genetically modified to increase the level of the amino acid lysine in the grain for animal feed, primarily for poultry and swine. Poultry and swine diets based on maize grain are usually supplemented with lysine. The use of LY038xMON810 as a feed ingredient is expected to reduce or eliminate the need for lysine supplementation. The maize line LY038xMON810 contains the *cordapA* gene from *C. glutamicum*.

The *cordapA* gene codes for the lysine-insensitive dihydrodipicolinate syntase (DHDPS), a regulatory enzyme in the lysine biosynthetic pathway. This enzyme mediates a critical rate-limiting step for lysine biosynthesis in plants and bacteria. DHDPS catalyzes the condensation of L-aspartate-β-semialdehyde and pyruvate, resulting in the synthesis of 2,3-dihydrodipicolinate, a substrate in the lysine metabolic pathway (8). DHDPS isolated from maize (mDHDPS) is highly susceptible to lysine feedback inhibition. Since the cDHDPS, encoded by *cordapA* and originating from *C. glutamicum*, is less sensitive to feedback inhibition than the native mDHDPS, its expression in maize LY038 results in elevated levels of free lysine accumulating in the grain of LY038 compared to those typically found in conventional maize.

In maize, the mDHDPS protein is synthesized in the cytoplasma and contains a chloroplast transit peptide (CTP) that directs the protein into the chloroplasts. To direct the introduced cDHDPS to the chloroplasts, the CTP from the mDHDPS was fused to the coding region of the cDHDPS protein.

Properties of the introduced genes conferring insect resistance

LY038xMON810 also expresses the *cry1Ab* gene derived from *B. thuringiensis* (subsp. *Kurstaki*). *cry1Ab* encodes an δ-endotoxin, which is lethal to insects of the Lepidopteran order, including larvae of the European corn borer *(Ostrinia nubilalis)* and the pink borer *(Sesamia cretica).* The δ-endotoxin selectively binds to receptors located in the midgut of susceptible insects (9). After this binding to receptors, the gut is perforated, enabling enterobacteria from the midgut to enter the body, causing the insect to die from poisoning within 48 to 120 hours (10).

The larvae of the European corn borer cause severe damage to corn crops by feeding on the stalks and creating boreholes. This results in weakened plants, eventually causing the plant to fall over. The damaged plants are also more susceptible to molds and rot. Furthermore, larvae can feed on the kernel causing a reduction of grain quality. The European corn borer is a pest insect in the United States and Canada. In the Netherlands however, this insect species is not of agronomic interest because the crop consists mainly of fodder maize. Together with the fodder maize, the pupae of the corn borer are chopped during harvesting; therefore, the corn borer population is not able to establish itself. In addition, the climate in the Netherlands is not optimal for the European corn borer.

Molecular analysis

The applicant has sufficiently proven that the hybrid LY038xMON810 contains one copy of the *cordapA* and the *cry1Ab* insert.

As mentioned before, COGEM assessed the risks of LY038. COGEM stated that there was lack of information regarding the molecular characterization. It was not sufficiently proven that the flanking regions of the insert represent the actual integration site in the genome or consist of co-integrated DNA. Furthermore, the applicant did not underpin its statement that the flanking sequences consist of genomic maize DNA. It was not clear whether DNA was co-integrated during the transformation. COGEM was of the opinion that missing data should be provided.

Advice

The present application concerns the commercial import and processing of maize line LY038xMON810 for use in food and feed. LY038xMON810 expresses the genes *cordapA* and *cry1Ab*, resulting in a higher lysine content in the grain and resistance to certain insects of the lepidopteran order.

Recently, COGEM issued an advice on maize line LY038. COGEM concluded that missing data concerning the molecular characterisation should be provided before a final opinion can be formed. Awaiting these results, COGEM can not give a positive advice on the maize line LY038xMON810.

References

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