Import of genetically modified maize LY038 with a higher lysine content

COGEM advice CGM/070504-01

This notification concerns the import and processing of the genetically modified maize line LY038 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. COGEM did not advise on such a maize line before. In 2006, an application was granted for cultivation of LY038 in the USA and Canada

In the Netherlands, no wild relatives of maize are present and establishment of maize plants in the wild has never been observed. There are no reasons to assume that the inserted traits will increase the potential of the maize line to establish feral populations. In addition, the appearance of volunteers is very rare under Dutch conditions.

COGEM points out that there is a lack of information regarding the molecular analysis. In particular incomplete data are presented on the analysis of the flanking sequences. It is not sufficiently proven that the flanking regions of the insert represent the actual integration site in the genome or consist of co-integrated DNA. Moreover, the applicant does not underpin its statement that the flanking sequences consist of genomic maize DNA.

COGEM is 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 can not be sufficiently substantiated. Therefore, COGEM is of the opinion that 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 LY038 for use in feed and food. Cultivation of LY038 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 cultivation in the USA and Canada (1).

The maize line is modified by the introduction and expression of the gene *cordapA* conferring a higher lysine content. COGEM did not advise on this type of genetically modified maize before.

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 (2-3). According to

literature, pollen viability varies between 30 minutes and 9 days (3-5). 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 (2). Establishment of maize plants in the wild has never been observed in the Netherlands. Also in warmer regions of Europe, this is not likely, even after warming by climate change the next decades.

Molecular characterisation

The genetically modified maize line LY038 was produced by particle acceleration and by means of the *cre/lox* recombination system. The DNA fragment used in particle acceleration contains two (*cordapA* and *nptII*) gene cassettes. The *nptII* gene cassette was located between two *loxP* sequences to allow for its subsequent removal by the *cre-lox* recombination system. The fragment used in the transformation was introduced into callus tissue originating from the maize inbred line H99. Maize plants containing the *cordapA* and *nptII* genes regenerated from callus tissue were crossed with plants expressing the Cre recombinase protein. The resulting hybrid underwent excision of the *nptII* gene cassette flanked by the *loxP* sites. The circular *nptII* gene cassette, as well as the Cre recombinase, was subsequently segregated away from plants containing the *cordapA* cassette through additional breeding, which resulted in LY038. Consequently, the DNA inserted in LY038 should contain only the *cordapA* gene cassette.

An overview of the introduced sequences 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

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 LY038 as a feed ingredient is expected to reduce or eliminate the need for lysine supplementation. The maize line LY038 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 (6). 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.

Molecular analysis

In the opinion of COGEM, the applicant has proven by Southern blot analysis that no backbone sequences or sequences from the *ntpII* gene cassette, located between the loxP sites, are present in LY038.

Digestion with Nde1, which does not cut in the entire insert, followed by Southern blot, should reveal the number of copies of the *cordapA* gene cassette. The copy number was investigated with a number of probes, spanning the entire insert. All blots reveal the expected fragments indicating the integration of a single insert. In a few cases, background hybridizing bands were present, most likely from endogenous homologs. COGEM states that hybridization experiments can never totally exclude the presence of

partial DNA fragments elsewhere in the genome. However, the applicant also performed a PCR analysis which confirmed the results from the Southern blot that only one copy of the *cordapA* gene cassette is present in the LY038 maize line.

The applicant performed bioinformatic and sequence analyses. Amongst others, the six reading frames were analysed for the presence of ORFs spanning the junction of the insert and the genomic DNA. All putative proteins encoded by the identified putative ORFs were compared with known sequences in databases. None of them showed homology with known toxins and allergens. Although the applicant states that 1781 nucleotides on the 5' and 667 nucleotides on the 3' flanking sequences of the insert were analysed and confirmed to be genomic maize DNA, it is not substantiated with data. Furthermore, COGEM is of the opinion that it is not sufficiently proven that the flanking regions of the insert represent the actual chromosomal integration site or consist of co-integrated DNA. Analysis by COGEM of the 5' and 3' flanking sequences shows that in all probability, a rearrangement did take place. A PCR analysis of the non-transgenic line could resolve whether the 5' and 3' flanking sequences are contiguous in the genome. Thus indicating the flanking regions representing the actual integration site in the genome.

COGEM is 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 can not be substantiated. Therefore, COGEM is of the opinion that the applicant has to provide the missing data. In absence of the data, authorisation should not be granted.

Advice

The present application concerns the commercial import and processing of maize line LY038 for the use in food and feed. LY038 expresses the gene *cordapA*, resulting in a higher lysine content in the grain.

There are no wild relatives of maize in the Netherlands and the appearance of volunteers is very rare under Dutch conditions. Furthermore, there are no reasons to assume that the inserted traits will increase the now absent potential of the maize line to run wild.

In the opinion of COGEM, it is sufficiently proven that no backbone sequences and that only one copy of the *cordapA* gene cassette, are present in LY038. However the analysis of the flanking regions was not adequate. It can not be stated with certainty that no DNA had co-integrated near the flanking regions. Moreover, the statement that the flanking regions consist of genomic maize DNA, is not substantiated with data.

COGEM is 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 can not be sufficiently substantiated. Therefore, COGEM is of the opinion that the applicant has to provide the missing data before a final opinion can be formed.

References

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