Import of genetically modified maize 59122 x 1507 x NK603

COGEM advice CGM/070817-01

Summary

This notification concerns the import and processing of the genetically modified maize line 59122 x 1507 x NK603 for use in feed and food. Cultivation is not part of the application. The hybrid maize line harbors two pat genes, a cp4epsps gene and three cry genes; cry34Ab1, cry35Ab1 and cry1F. The pat and cp4epsps genes confer tolerance to herbicides containing glufosinate-ammonium and glyphosate, respectively. The cry34Ab1 and cry35Ab1 genes are responsible for resistance to certain coleopteran insects such as the corn rootworm (Diabrotica virgifera) while cry1F provides the plant with resistance trait to certain lepidopteran insects such as the European corn borer (Ostrinia nubilalis).

Previously, COGEM advised positively on the import of maize lines 59122, 1507 and NK603. COGEM has also given a positive advice on the cultivation of maize line 1507 and NK603 as well as on the cultivation of the hybrid maize line 1507 x NK603.

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 is of the opinion that interactions between recombinant gene products are not to be expected. The applicant has sufficiently proven that no toxic or allergenic products are formed as a result of the modification of maize line 59122 x 1507 x NK603. Based on these considerations, COGEM is of the opinion that the import of maize line 59122 x 1507 x NK603 poses a negligible risk to human health and the environment.

Introduction

The present notification by Pioneer Hi-Bred International, Inc. concerns the import and processing of the hybrid maize line 59122 x 1507 x NK603 for use in feed and food.

The maize line is produced by traditional crossing of maize lines 59122, 1507 and NK603. Maize line 59122 expresses the *pat* gene conferring tolerance to glufosinate-ammonium containing herbicides and the two *cry* genes *cry34Ab1* and *cry35Ab1* conferring insect resistance to certain coleopteran insects such as the corn rootworm *(Diabrotica virgifera)*. Maize line 1507 expresses a *pat* gene as well, plus a *cry1F* gene which provides the resistance trait to lepidopteran insects such as the European corn borer *(Ostrinia nubilalis)*. Parental maize line NK603 expresses *cp4epsps*, conferring tolerance to glyphosate containing herbicides.

Previous COGEM advices

Previously, COGEM advised positively on the import of maize lines 59122, 1507 and NK603 (1, 2, 3). COGEM has also given a positive advice on the cultivation of maize lines 1507, NK603 and 1507 x NK603 (4, 5, 6).

Aspects of the crop

Maize (*Zea mays L.*) is a member of the grass family *Poaceae.* Maize is originating from Central America. Although insect pollination can not be completely excluded, maize is predominantly wind pollinated (7,8). According to literature, pollen viability varies between 30 minutes and 9 days (8, 9, 10). 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. Generally, only few kernels remain on the field after harvesting of fodder maize (7). Kernels exhibit no dormancy, resulting in a short persistence under Northern European climate conditions.. Establishment of maize plants in the wild has never been observed in the Netherlands.

Molecular characterization

The genetically modified maize line 59122 x 1507 x NK603 was produced by crossing three parental maize lines 59122, 1507 and NK603. The characterization of these parental lines will be briefly discussed. A more detailed description can be found in previous COGEM advices on the single parental maize lines (1,2,3).

Description of the introduced genes

Maize line 59122 expresses the *cry34Ab1*, *cry35Ab1* and a *pat* gene. Besides these three genes, necessary regulatory components to regulate gene expression were inserted as part of the vector.

Maize line 1507 was genetically modified by insertion of the *cry*1F and the *pat* gene and the regulatory components necessary.

The genetically modified maize line NK603 contains two *cp4 epsps* expression cassettes. The *cp4epsps* gene was isolated from the CP4 strain of the soil bacterium *Agrobacterium tumefaciens.*

Properties of the introduced genes conferring herbicide tolerance

The hybrid maize line 59122 x 1507 x NK603 confers two different genes for herbicide tolerance; the *pat* gene and the *cp4epsps* gene.

Cp4epsps which was inserted in maize line NK603 encodes CP4EPSPS proteins possessing high tolerance to glyphosate containing herbicides. EPSPS is a natural occurring enzyme involved in the biosynthesis of aromatic amino acids. In non-

transgenic maize lines, glyphosate acts by binding to, and thus inhibiting the function of naturally occurring EPSPS. Consequently, aromatic amino acids are no longer formed, leading to plant death. In contrast, CP4EPSPS is not affected by glyphosate because of a reduced binding affinity. NK603 expresses the gene *cp4epsps*, resulting in a high tolerance to glyphosate. The application of this herbicide will not cause death of the maize line, because the plant is still able to produce aromatic amino acids (11). Epsps proteins are active in the chloroplasts of a plant cell. A *ctp2* signal peptide is fused to the *epsps* transgene, resulting in the transport of the transgenic EPSPS protein to the chloroplast (12).

Maize line 59122 as well as maize line 1507 are genetically modified by the introduction of a *pat* gene, encoding the phosphinothricin acetyltransferase enzyme (PAT). Expression of PAT confers tolerance to glufosinate-ammonium herbicides (13).
The active ingredient in glufosinate-ammonium herbicide is L-phosphinothricin (L-PPT), which binds to glutamine synthetase in plants. The detoxification of excess ammonia is thereby prevented, leading to plant death. Maize line 59122 and 1507 express the *pat* gene which catalyses the conversion of L-PPT to an inactive form, which does not bind glutamine synthetase. The application of glufosinate-ammonium herbicides to maize line 59122 and 1507 will therefore be ineffective since ammonia is detoxified (14).

Properties of the introduced genes conferring insect resistance
Maize line 59122 was genetically modified with the *cry34ab1* and the *cry35Ab1* gene derived from *Bacillus thuringiensis* (subsp. *kumamotoensis*). These genes act together to control certain coleopteran insect pests, among others the larvae of the Western corn rootworm (*Diabrotica virgifera virgifera*), Northern corn rootworm (*Diabrotica barberi*) and the Southern corn rootworm (*Diabrotica undecimpunctata howardi*). The genes *cry34Ab1* and *cry35Ab1* have a synergistic effect. The *cry34Ab1* gene confers an average resistance to the corn rootworm, while *cry35Ab1* alone confers no resistance at all. Expression of both genes in the same plant however, results in a maximum resistance against the corn rootworm. The Cry34Ab1 and Cry35Ab1 proteins are δ-endotoxins which selectively bind to receptors located in the midgut of susceptible insects. After binding, the gut is perforated, enabling enterobacteria from the midgut to enter the body, causing the insect to die within 48 to 120 hours (15).

The corn rootworm is an economically important pest insect, which causes major crop losses. Larvae of this insect feed on maize roots, resulting in the interference of the plant's ability to absorb water and nutrients and in the reduction of the stability of the plant. As a consequence, damaged plants may lodge, making harvesting difficult.

Corn rootworm was accidentally introduced in the mid-nineties in Bosnia, presumably by military air traffic, and established itself shortly after introduction. The pest is still spreading at a rate of about 40 km per year, but is infamous for rapid spread over large distances by (air) traffic. In 2003 this insect was first discovered near the airport of Schiphol in the Netherlands, but eradicated successfully. If the corn rootworm is able to establish and spread itself in the Netherlands, large crop damage can be expected (16).

Maize line 1507 expresses cry gene *cry1F*. *Cry1F* encodes another 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).* Cry1F has the same physical effect on the insects as Cry34Ab1 and Cry35Ab1.

The larvae of the European corn borer cause severe damage to corn 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 which reduces the 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

Previously, the molecular aspects of parental maize line 59122 as well as 1507 and NK603 have been thoroughly analyzed (1,2,3). COGEM is of the opinion that the molecular analysis of the three parental maize lines 59122, 1507 and NK603 was sufficient and it is unlikely that toxic or allergenic products are formed as a consequence of the modifications.

Advice

The present application concerns the commercial import and processing of maize line 59122 x 1507 x NK603 for the use in food and feed. Maize line 59122 x 1507 x NK603 expresses the genes *pat, cp4epsps*, *cry34Ab, cry35Ab1 and cry1F*, providing the plant with a herbicide tolerance trait as well as resistance to certain coleopteran and lepidopteran insects. In the past, COGEM advised positively on the import of maize lines with these traits and hybrid maize lines with a combination of these traits.

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 traits will enable the maize line to run wild.

In the opinion of COGEM, the molecular analysis for 59122 x 1507 x NK603 is adequate. In addition, maize line NK603 and 1507 as well as hybrid maize line NK603 x 1507 have already been commercially grown and consumed for several years in the U.S.A and in several other countries without any reports of adverse effects.

In view of these considerations, COGEM is of the opinion that the proposed import and processing of maize line 59122 x 1507 x NK603 poses a negligible risk to human health and the environment.

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