import and processing of maize Bt11xGA21

COGEM advice CGM/080417-01

Summary

The present application by Syngenta Seeds S.A.S. (file EFSA/GMO/UK/2007/49) concerns import and processing for use in feed and food of the genetically modified maize line Bt11xGA21. Cultivation is not part of this application.

Maize line Bt11xGA21 was obtained by conventional cross-breeding of the two parental lines. Previously, COGEM issued positive advices on import and processing, and on cultivation of maize line Bt11. COGEM also advised on import and processing of maize line GA21. COGEM considered the ecological risks associated with the import and processing of maize line GA21 negligible.

The hybrid maize line Bt11xGA21 contains the cry1Ab gene conferring resistance to certain lepidopteran insects. In addition, this maize line contains the pat and mepsps genes, resulting in tolerance to glyphosate and glufosinate ammonium containing 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 genes introduced in Bt11xGA21 cannot spread to closely related species since wild relatives of maize are not present in Europe.

In view of the above, COGEM is of the opinion that incidental spillage of Bt11xGA21 poses negligible risks to the environment. Therefore, COGEM considers the risks associated with import and processing of maize line Bt11xGA21 negligible. However, COGEM questions some aspects of the provided general surveillance plan.

Introduction

The present application by Syngenta S.A.S., file EFSA/GMO/UK/2007/49, concerns the import and processing of maize line Bt11xGA21 for use in feed and food. Maize line Bt11xGA21 was obtained by conventional cross-breeding of the two parental lines Bt11 and GA21. The hybrid maize line Bt11xGA21 contains the *cry1Ab, pat* and *mepsps* genes, which are constitutively expressed. As a result Bt11xGA21 is resistant to certain lepidopteran insects and tolerant to glyphosate and glufosinate ammonium containing herbicides.

Previous COGEM advices

In 1997, COGEM issued a positive advice on import and processing of maize line Bt11 (1). In addition, a positive advice on cultivation of this maize line has been issued in 2005 (2).

COGEM has also advised on maize line GA21 and concluded that the ecological risks associated with the import and processing of this maize line are negligible (3, 4).

Aspects of the crop

Maize (*Zea mays*) is a member of the *Poaceae* family (grasses). Maize was domesticated in Central America and is nowadays cultivated throughout the world (5). In Europe, hybridization with other species cannot occur as wild relatives of maize are not present in Europe (5). The appearance of volunteers is very rare under Dutch conditions. Grains exhibit no germination dormancy, resulting in a short persistence. Establishment of maize plants in the wild has never been observed in the Netherlands. Besides, observations outside the Netherlands indicate that feral maize populations do not occur in Europe.

Molecular characterization

Maize line Bt11xGA21 was produced by traditional cross-breeding of the two genetically modified parental maize lines Bt11 and GA21. The molecular characterization of these parental lines is discussed below.

Parental maize line Bt11:

Bt11 maize was generated by transformation of *Z. mays* protoplasts using a *Not*I restriction fragment which contains *cry1Ab* and *pat* gene cassettes. Besides these gene cassettes a 1.1 kb fragment of vector sequence is present upstream of the *cry1Ab* gene cassette. This fragment contains the ColE1 *ori*, the origin of replication that permits replication of plasmids in *Escherichia coli*, but which is not functional in plants.

Maize line Bt11 contains a single DNA insertion with one copy of the *Not*I restriction fragment.

The *cry1Ab* gene cassette consists of the following elements:

- 35S promoter, derived from *Cauliflower mosaic virus* (CaMV)
- IVS6-ADH1 intron, intervening intron sequence 6 derived from the alcohol dehydrogenase 1 (*adh1*) gene of maize
- truncated *cry1Ab* gene, derived from *Bacillus thuringiensis* var. *kurstaki* HD-1, truncated at the 3' end and modified to enhance expression in plants. The Cry1Ab protein confers resistance to certain lepidopteran insects
- NOS terminator, derived from the nopaline synthase (*nos*) gene of *Agrobacterium tumefaciens*

The *pat* gene cassette consists of the following elements:

- 35S promoter, derived from CaMV
- IVS2-ADH1 intron, intervening intron sequence 2 derived from the alcohol dehydrogenase 1 (*adh1*) gene of maize
- *pat* gene, derived from *Streptomyces viridochromogenes* strain Tu494 and codon-optimized to enhance expression in maize. The *pat* gene encodes phosphinothricin acetyl transferase which confers resistance to glufosinate ammonium containing herbicides
- NOS terminator, derived from the nopaline synthase (*nos*) gene of *A. tumefaciens*

The regions that flank the insert in maize line Bt11 are primarily homologous to the *Z. mays* 180 bp knob-associated tandem repeat. Knobs are components of the maize heterochromatin, a class of chromatin which is not expressed. Therefore, it can be concluded that the insert does not disrupt an endogenous maize gene.

Parental maize line GA21:

GA21 maize was produced by microprojectile bombardment of maize suspension cells using a *Not*I restriction fragment.

The *Not*I restriction fragment contains the following elements:

- ract1 promoter, first intron and exon, derived from the rice actin 1 (*ract1*) gene
- optimized CTP, N-terminal chloroplast transit peptide (CTP) based on CTP sequences from sunflower and maize
- *mepsps* gene, modified 5-enolpyruvylshikimate-3-phosphate synthase gene from maize
- NOS terminator, derived from the nopaline synthase (*nos*) gene of *A. tumefaciens*

Maize line GA21 contains a single DNA insertion with six (partial) copies of the *Not*I restriction fragment. Copy 1 contains the ract1 promoter that has a deletion of 696 bp, the ract1 first intron and exon, the optimized CTP, the *mepsps* gene and the NOS terminator. Copies 2, 3 and 4 are intact versions of the *Not*I restriction fragment. Copy 5 contains the ract1 promoter, first intron and exon, the optimized CTP and the first 288 bp of the *mepsps* gene which ends in a stop codon. The NOS terminator is not present in copy 5. Copy 6 contains the ract1 promoter and a truncated ract1 first exon. No other elements of the *Not*I fragment are present in copy 6.

Northern Blot Analysis showed that only the full length CTP-*mepsps* transcript was present. In addition, Western Blot Analysis detected only the full-length mEPSPS protein. These results indicate that no truncated EPSPS fragment is expressed in GA21.

Analysis of the region that flanks the 5' end of copy 1 showed that this region is homologous to maize chloroplast DNA. The insert appears to have disrupted a protein homologous to a cytochrome C biogenesis protein. The function of this disrupted protein will probably be compensated by a functional cytochrome C biosynthesis gene in the maize chloroplast genome. Analysis of the region that flanks the 3' end of copy 6 showed that this region was homologous to several maize sequences in the NCBI nucleotide database. This 3' region probably represents repetitive sequence elements present in the maize genome.

In addition, the applicant analyzed the above-described flanking regions for the presence of putative open reading frames (ORFs). None of the identified putative ORFs spanning or near the junction sites was homologous to allergenic or toxic proteins.

Properties of the introduced genes conferring insect resistance

Maize line Bt11 was genetically modified by insertion of the *cry1Ab* gene. The *cry1Ab* gene encodes a δ-endotoxin specific for certain lepidopteran insects, e.g. the European corn borer (*Ostrinia nubilalis*). The δ-endotoxins are solubilized in the midgut of susceptible insects and are activated by midgut proteases to release a toxin fragment. The toxin fragment binds to specific receptors on the epithelial surface of the midgut. Subsequently, pores are formed in the membranes of the gut cells of the insect, enabling midgut bacteria to enter the body cavity, which leads to septicemia and death (6).

Properties of the introduced genes conferring herbicide tolerance

In addition to the *cry1Ab* gene, the *pat* gene was inserted in maize line Bt11. The *pat* gene encodes phosphinothricin-N-acetyl transferase (PAT). This protein acetylates L-phosphinothricin, the active isomer of glufosinate ammonium.

In non-transgenic plants glufosinate ammonium inhibits the activity of glutamine synthetase, an enzyme necessary for the production of glutamine and for ammonia detoxification. The application of glufosinate ammonium leads to reduced glutamine and increased ammonia levels in non-transgenic plants (7). Photosynthesis is inhibited and eventually the plant dies (8).

In maize line Bt11 the PAT protein acetylates L-phosphinothricin, the active isomer of glufosinate ammonium. The resulting compound N-acetyl-L-phosphinothricin does not inhibit the activity of glutamine synthetase (7). As a result maize line Bt11 is tolerant to L-phosphinothricin and thus to glufosinate ammonium containing herbicides.

Maize line GA21 expresses the *mepsps* gene which encodes a modified 5-enolpyruvylshikimate-3-phosphate synthase 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. Maize

line GA21 expresses a modified EPSPS protein, which is not inhibited by glyphosate, and is therefore tolerant to glyphosate containing herbicides (9).

Molecular analysis

In the past, COGEM assessed the molecular analysis of parental maize lines Bt11 and GA21. The molecular analysis of maize line Bt11 was considered adequate (2). However, in the opinion of COGEM the molecular analysis of maize line GA21 was incomplete (3). The data on the region that flanks the 5' end of copy 1 of the insert present in maize line GA21 was partially lacking and therefore it could not be excluded that new ORFs were created due to the insertion. Theoretically, these putative ORFs could give rise to potentially allergenic or toxic proteins. Recently, COGEM abstains from advices on the potential risks of incidental consumption in case a food/feed assessment is already carried out by other organizations. Therefore, considerations of COGEM regarding previous objections concerning the molecular analysis of maize line GA21 in view of incidental consumption are not included (3). The outcome of the assessment of consumption by other organizations was not known at the moment of completion of this advice.

With regard to the above, COGEM is of the opinion that the molecular analysis of hybrid maize line Bt11xGA21 does not indicate that import and processing of this line would pose a risk to the environment.

Environmental risk assessment

During the long process of domestication, maize has lost the ability to survive in the wild. In addition, maize needs human intervention to disseminate its seed. Maize kernels exhibit no dormancy and can only survive within a narrow range of climatic conditions. Furthermore, maize is very sensitive to weed competition and cannot persist as a weed (5, 10). In the Netherlands, volunteers are rarely found and establishment of maize plants in the wild has never been observed. There are no reasons to assume that the introduced traits will increase the potential of Bt11xGA21 to establish feral populations in case of incidental spillage.

General surveillance plan

Several organizations representing trade organizations that import or use viable maize, e.g. COCERAL, UNISTOCK and FEDIOL, are mentioned in the general surveillance plan. According to the applicant these organizations are 'well-placed' to detect unanticipated effects on human health or the environment. However, information concerning their expertise in the environment is not given. In addition, it is unclear whether these organizations have agreed to cooperate in the general surveillance of Bt11xGA21. As stated in previous advices, COGEM is of the opinion that the applicant should ascertain that information on potential adverse effects is obtained. In addition, COGEM would prefer independent organizations which have expertise on the

environment 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).

Furthermore, according to the applicant indirect or delayed effects will be reported at the stage of re-evaluation or at the end of a given consent. As stated before, in COGEM's opinion all observed effects, including indirect and delayed effects, should be reported annually.

Advice

COGEM has been asked to advice on import and processing for use in feed and food of hybrid maize line Bt11xGA21.

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 the expression of the *cry1Ab, pat* and *mepsps* genes in Bt11xGA21 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 line Bt11xGA21 poses negligible risks to the environment. Therefore, COGEM considers the risks associated with import and processing of maize line Bt11xGA21 negligible. However, COGEM questions some aspects of the provided general surveillance plan.

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