

Renewal of authorization for import and processing of maize Bt11

COGEM advice CGM/080523-02

Summary

The present application by Syngenta Seeds S.A.S. (file EFSA/GMO/RX/Bt11) concerns the renewal of the authorization for import and processing of the genetically modified maize line Bt11. Cultivation is not part of this application.

Previously, COGEM issued positive advices on import and processing, and on cultivation of maize line Bt11. The maize line contains the cry1Ab gene conferring resistance to certain lepidopteran insects. In addition, Bt11 contains the pat gene, resulting in tolerance to 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 maize plants 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.

In view of the above, COGEM is of the opinion that incidental spillage of Bt11 poses negligible risks to the environment. Therefore, COGEM considers the risks associated with import and processing of maize line Bt11 negligible. However, COGEM questions some aspects of the provided general surveillance plan. Furthermore, COGEM has some remarks on the fact that the sequence data in the current application differs from the information present in previous applications.

Introduction

The present application by Syngenta S.A.S., file EFSA/GMO/RX/Bt11, concerns the renewal of the authorization for import and processing of maize line Bt11. Maize line Bt11 has been cultivated in the USA and Canada since 1997. In 1998 maize line Bt11 was authorized for food and feed use in the European Union. Maize line Bt11 contains the cry1Ab and pat genes, which are constitutively expressed. As a result Bt11 is resistant to certain lepidopteran insects and tolerant to 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).

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 (3). In Europe, hybridization with other species cannot occur as wild relatives of maize are not present in Europe (3). The appearance of volunteers is very rare under Dutch conditions. Grains exhibit no germination dormancy, resulting in a short persistence. Feral populations of maize have never been observed in the Netherlands or in Europe.

Molecular characterization

Bt11 maize was generated by transformation of *Z. mays* protoplasts using a *NotI* restriction fragment which contains *cryIAb* and *pat* gene cassettes. Besides these gene cassettes in the restriction fragment a 1.1 kb fragment of vector sequence is present upstream of the *cryIAb* 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 *NotI* restriction fragment.

The *cryIAb* 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 *Z. mays*
- truncated *cryIAb* gene, derived from *Bacillus thuringiensis* var. *kurstaki* HD-1, truncated at the 3' end and modified to enhance expression in plants
- 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 *Z. mays*
- *pat* gene, derived from *Streptomyces viridochromogenes* strain Tu494 and codon-optimized to enhance expression in maize
- NOS terminator, derived from the nopaline synthase (*nos*) gene of *A. tumefaciens*

The regions that flank the insert in maize line Bt11 are homologous to the *Z. mays* 180 bp knob-associated tandem repeat. Knobs are components of the maize heterochromatin and are therefore strongly repressed.

Properties of the introduced genes conferring insect resistance

Maize line Bt11 was genetically modified by insertion of the *cryIAb* gene. The *cryIAb* 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 (4).

Properties of the introduced genes conferring herbicide tolerance

In addition to the *cryIAb* 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 (5). Photosynthesis is inhibited and eventually the plant dies (6).

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 (5). As a result maize line Bt11 is tolerant to L-phosphinothricin and thus to glufosinate ammonium containing herbicides.

Molecular analysis

The current application for the renewal of the authorization for import and processing of maize line Bt11 contains updated information concerning the molecular characterization. The insert and the maize genomic regions that flank the insert were resequenced and the obtained sequence was compared with the previously reported sequence. Eight nucleotides were different from the previously reported sequence. Four of these nucleotides are located in the intergenic region of the insert and two of these nucleotides are present in the maize genomic region that flanks the 3' end of the insert. In addition, a different nucleotide was identified in both terminators of the insert. The observed nucleotide change was identical for both terminators.

The differences between the current and the previously reported sequence could result from naturally occurring mutations or may represent flaws in previous analyses. However, the identical nucleotide changes observed in both terminators indicates that these observed differences result from flawed analyses.

Four of the changed nucleotides are located in non-coding regions and two of the nucleotide changes are situated in the maize heterochromatin. The applicant states that

the Cry1Ab and PAT proteins are expressed correctly and concludes that the nucleotide changes in the terminator do not have an effect on its function.

COGEM is of the opinion that there are no reasons to assume that the observed changes in nucleotides will lead to different characteristics of the maize plants. Therefore, the observed differences will not change the outcome of previous environmental risk assessments.

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. The monitoring plan indicates that these organizations could assist in general surveillance by reminding their members to perform monitoring and by collecting data from their members. According to the applicant these trade 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 Bt11. Furthermore, a monitoring protocol which describes how monitoring should be conducted is not supplied by the applicant.

As stated in previous advices, COGEM is of the opinion that the applicant should ascertain that information on potential adverse effects is obtained (8, 9, 10). 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).

Advice

COGEM has been asked to advice on the renewal of the application for import and processing for use in feed and food of maize line Bt11. This maize line is imported in Europe since 1998 and has a history of safe use.

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* and *pat* genes in Bt11 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 the opinion of COGEM there are no reasons to assume that the different nucleotides observed between the current and the previously reported sequence will lead to different characteristics of the maize plants.

Based on the considerations put forward in this advice, COGEM is of the opinion that incidental spillage of maize line Bt11 poses negligible risks to the environment. Therefore, COGEM considers the risks associated with import and processing of maize line Bt11 negligible. However, COGEM questions some aspects of the provided general surveillance plan.

Additional remark

The original sequence data of maize line Bt11 was revised and appeared to contain base pair changes. COGEM emphasizes that risk assessments depend on the information provided by the applicant. Therefore, this information is of paramount importance for the European legislation regarding authorization of GMOs. A proper risk assessment can only be carried out if adequate and correct information is supplied. If such information is not present, the risk assessment may be hampered. Furthermore, if the information provided turns out to be incorrect, this may damage the confidence of the competent authorities assessing this information. Moreover, this also applies to the confidence of European citizens in (future) consumer products containing GMOs and indirectly in their confidence in the European and national governments.

References

1. COGEM (1997). Advies C/GB/96/M4-01 betreffende het in het handelsverkeer brengen van genetisch gemodificeerde maïs waarin het *cry-IA(b)* gen (Bt-toxine) en het *pat* gen tot expressie komen (CGM/970204-06)
2. COGEM (2005). Assessment of an EFSA opinion on the cultivation of Bt11 maize (CGM/050816-01)
3. OECD (2003). Consensus document on the biology of *Zea mays* subsp. *mays* (Maize)
4. Broderick NA, Raffa KF and Handelsman J (2006). Midgut bacteria required for *Bacillus thuringiensis* insecticidal activity. Proceedings of the National Academy of Science USA 103, 15196-15199
5. OECD (1999). Consensus document on general information concerning the genes and their enzymes that confer tolerance to phosphinothricin herbicide
6. OECD (2002). Module II: Phosphinothricin
7. Crop Protection Compendium (2004). *Zea mays* (maize). CD-ROM edition, © Cab International 2004, Nosworthy way, Wallingford, UK
8. COGEM (2008). Import and processing of maize Bt11xGA21 (CGM/080417-01)
9. COGEM (2008). Import and processing of maize MIR604xGA21 (CGM/080521-02)
10. COGEM (2008). Import and processing of maize Bt11xMIR604 (CGM/080521-03)
11. COGEM (2005). Post market monitoring van genetisch gemodificeerde gewassen in Nederland (CGM/050414-03)